HISTOPATHOLOGICAL ANALYSIS OF LUNG TUMORS AND STUDY OF EXPRESSION OF EGFR WITH IMMUNOHISTOCHEMICAL MARKERS

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I, **Dr. C.Sofia Tamilarasi,** solemnly declare that the dissertation titled **"HISTOPATHOLOGICAL ANALYSIS OF LUNG TUMORS AND STUDY OF EXPRESSION OF EGFR WITH IMMUNOHISTO CHEMICAL MARKERS"** is a bonafide work done by me at Department of Pathology, Madurai Medical College & Government Rajaji Hospital, Madurai during the period from July 2015 to August 2017.

I also declare that this bonafide work or a part of this work was not submitted by me or any other for any reward, degree and diploma to any university, board either in India or abroad.

This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, towards partial fulfillment of requirement for the reward of M.D. Degree in Pathology.

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CONTENTS

INTRODUCTION

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Lung carcinoma is one of the frequent and leading causes of cancer related mortality worldwide. The highest incidence is in central Europe & Eastern Asia with 12.7% of worlds total cancer incidence [(1)].Incidence rate is low in Africa. In India, Lung carcinoma is more common and severe among males especially smokers. The incidence rate is relatively low among Indian women[(3)].

Lung carcinomas are classified clinically mainly into two subtypes

1. Non Small Cell Carcinoma (75 to 85 %)
2. Small Cell Carcinoma (10 – 25%)

Non-small cell lung cancer (NSCLC) accounting for the most frequent type. Majority of the patients with NSCLC have locally advanced or metastatic disease at the time of diagnosis. For a long period, chemotherapy was the only choice of therapeutic strategy for patients with this malignancy, but the prognosis is very poor [(9)]. With a median survival time of only 8–10 months and 5-year survival rate less than 20% [(10)].

Non Small Cell Carcinoma further histologically sub classified into,

- Adenocarcinoma (40 – 45%)
- Squamous Cell Carcinoma (20 – 25%)
- Large cell or undifferentiated carcinoma (10 – 15 %)
- Adenosquamous carcinoma
- Sarcomatoid carcinoma– less common.

With the recent therapeutic advancements, due to availability of personalized medicine and targeted therapies , it is essential to identify the specific subtypes of non small cell subtypes so that treatment options are implemented. The discovery of specific genealterations, particularly those responding to tyrosine kinase inhibitors relatively benefits the patients with specific mutations .

The most frequent molecular alterations in Lung carcinomas are epidermal growth factor receptor (EGFR), K-RAS genes, rearrangements of anaplastic lymphoma kinase

(ALK)genes. The expression of EGFR genes correlates with better response to tyrosine kinase inhibitors(TKI) like Gefitinib and Erlotinib. More recently Afatinib, a TKI drug approved for the treatment of patients with lung adenocarcinoma, have been shown to significantly extend progression-free and overall survival in patients those harbor activating EGFR mutations. EGFR expression can be identified by IHC, PCR and FISH techniques.

K- RAS mutation expressed in 15 – 30 % of lung adenocarcinomas has resistance to Tyrosine kinase inhibitors. The other mutations for targeted therapy are BRAF, FGFR, HER 2, & ROS 1.

Immunohistochemistry has become an indispensable ancillary tool for the accurate classification of pulmonary neoplasms necessary for therapeutic decisions and predicting prognostic outcome in the era of personalized medicine. Diagnostic accuracy has significantly improved because of the

continuous discoveries of tumor-associated biomarkers and the development of effective immunohistochemical panels.

IHC done for EGFR has approximate sensitivity of 90 % and specificity of 88 %. It is estimated that IHC positive tumors show strong EGFR expression, whereas IHC negative tumors have low or no expression.

AIMS AND OBJECTIVES

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- To study the frequency of occurrence of lung tumors in small biopsies received from Government Rajaji Hospital, Madurai to the Department of Pathology, Madurai Medical College, Madurai.
- To study the age and sex related incidence in various Lung carcinomas.
- To assess the expression of Epidermal Growth Factor Receptor (EGFR) in Lung carcinomas by immunohistochemistry.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORICAL ASPECTS:

- In 1895 Rigid bronchoscope was introduced by Gustav Killian (1860-1921) in Germany. He is considered to be the "Father of bronchoscope".
- In 1904, Chevalier Jackson equipped the bronchoscope with an electric light source at the distal end and also added the suction channel.
- In 1960, Japanese physician Shigeto Ikeda replaced electric bulb with glass fibers and thus presented the first flexible bronchoscope at the International congress on diseases of the chest in Copenhagen.
- In1980s Asahi Pentax replaced the fibreoptic bundle with a charge coupled sensor,the video bronchoscope.

EMBRYOLOGY:

Lung development is divided into five stages, based on anatomic and histological characteristics. These are the embryonic, pseudoglandular, canalicular, saccular and alveolar stages[12]. Vascular and airway development are closely related. The conducting airways are formed in the embryonic and pseudoglandular stages, while gas exchange units characterized by vascularization and reduction of mesenchyme are formed in the canalicular, saccular and alveolar stages.

AIRWAY DEVELOPMENT:

During early organogenesis around 3 weeks after fertilization, the lungs begins as a diverticulum of endoderm in the ventral floor of the foregut , surrounded by an amorphous condensation of splanchnic mesoderm and it lengthens anterior to the esophagus[13].

By the fourth week of gestation, two lung buds form as distal outgrowth by repetitive nondichotomous branchings results in the formation of the primordial bronchial tree by the eighth week of gestation. By 17 weeks, the primitive structure of the conducting airways has been formed, it is referred to as the pseudoglandular stage.

The later stages of development (canalicular, 13–25 weeks; saccular , 24 weeks to birth; and alveolar, late fetal to the age of 8–10 years) are commited to the formation of the essential units of respiration, the acini and its maturation.

ANATOMY:

By the end of gestation, five well-defined lung lobes are present, three on the right (upper, middle, and lower lobes) and two on the left (upper and lower lobes).Each of the five primary lobar buds is invested with visceral pleura[14]. Each lobe in turn is composed of one or more segments, resulting in a total of 10 segments per lung, the right middle lobe and the lingula are analogous structures.

As gestation proceeds, airway branching continues to with each successive division, the leads to a progressive increase in airway volume and significant reduction in airway resistance in more distal lung.

HISTOLOGY:

The basic components of the lung parenchyma are bronchi, bronchioles (conducting airways) and the alveoli. The alveoli are lined by type I and type II pneumocytes; the latter produce surfactant [16] .The alveolar walls contain capillaries in which the basement membrane fuses with that of the alveolar epithelium to constitute a single alveolar capillary membrane.

The important cell types of the bronchial–bronchiolar epithelium are basal cells, neuroendocrine (Kulchitsky-type) cells [15], ciliated cells, serous cells, Clara cells, and goblet cells. Goblet and ciliated cells decrease in number as one proceed to the terminal bronchioles, whereas the number of Clara cells increases proportionally. Clara cells have a secretory function and represent the main progenitor cells after bronchiolar injury. Kulchitsky-type cells are part of the diffuse neuroendocrine system.[15]

EPIDEMIOLOGY

At the beginning of the 20th century, lung cancer was a relatively rare disease, but by the end of the century it had become the major cancer-related public health problem in the world [1]. In the 1950s, case–control studies strongly established the association between smoking and lung cancer [17]. Although tobacco had been widely smoked for several centuries, the rise of

lung cancer is due to availability at a lower cost, increased daily usage, and sustained exposure of the lungs to inhaled carcinogens.

ETIOLOGICAL FACTORS

SMOKING

Nearly 90% of all cases of lung cancer are caused by chronic exposure of the bronchial mucosa to carcinogens found in cigarette smoke. Case–control studies published in the 1950s by Wynder and Graham in the U. S, Doll and Hill in England proved the first strong scientific link.

Subsequently many epidemiologic studies have confirmed that the strongest determinants of lung cancer in smokers are the duration of tobacco use and the number of cigarettes smoked [(18)]. Passive, or secondhand, exposure to cigarette smoke can increase the risk of lung cancer by up to 25% Lifelong non-smokers account for 10–15% of patients with lung cancer.

The incidence of lung cancer is higher in female than male who never smokes. This gender disparity is due to increased susceptibility to lung cancer in women [(19)] or to a greater likelihood of exposure to passive cigarette smoke[(20)].

LUNG CANCER IN NEVER SMOKERS:

25% of lung cancer according to WHO estimation worldwide occurs in never smokers. This percentage is probably closer to 10% to 15% in Western countries. These cancers occur more commonly in women and most are

adenocarcinomas. Cancers in nonsmokers are more likely to have EGFR mutations, and almost never have KRAS mutations; TP53mutations are not uncommon, but occur less frequently than in smoking related cancers.

ENVIRONMENTAL POLLUTION:

Numerous environmental pollutants, have been associated with an increased risk of lung cancer, including radon, asbestos, air pollution, chromium, nickel, polycyclic aromatic hydrocarbons, and arsenic[(21)]. Each of these may account for a low percentage of lung cancers in non-smokers, but more commonly, exposure to these agents appears to act synergistically with tobacco carcinogens to increase the risk of lung cancer in smokers [(22)].

OCCUPATIONAL EXPOSURE

Asbestos is an independent lung carcinogen. In a retrospective cohort study published in 1955, Doll noted a 10-fold increased risk of lung cancer in asbestos textile workers [(31,32)]. Radon exposure in underground miners was connected to lung cancer risk in the early 1910s.

More recently, indoor radon exposure has been implicated as a risk factor for lung cancer, though the relative risk is much lower than that noted for miners [(34,35)]. However, it is estimated that indoor radon exposure may account for up to 15,000 lung cancer deaths [(37)].

ASSOCIATION WITH OTHER LUNG DISEASES:

Certain lung diseases are associated with an increased risk of lung cancer. The proven association is with chronic obstructive pulmonary disease (COPD) [(24)]. Both COPD and lung cancer are so highly related to cigarette use that studies have proved the effect of smoking on the causal link between COPD and lung cancer.

Interstitial lung disease and systemic sclerosis have also been reported as risk factors for lung cancer, due to chronic lung inflammation which may lead to genetic changes in bronchial epithelial and stromal cells [(30)].

FOOD HABITS:

Epidemiologic studies have implicated various dietary factors in the risk of lung cancer[(38)]. Diets rich in fruits and vegetables are associated with a lower incidence of lung cancer, and higher dietary intake and blood levels of betacarotene[(39)].Total carotenoids correlate with 30–80% lower risk of lung cancer, even after adjusting for smoking, age, and gender[(40)].

INFECTION:

Epstein-Barr virus (EBV) infection is associated with primary pulmonary lymphoepithelioma-like carcinoma (LELC). EBV infection is relatively common in Chinese, Taiwanese, Japanese and Eskimo populations. The EBV-associated carcinoma morphologically resembles the EBV-associated

undifferentiated nasopharyngeal carcinoma and probably representing a clonal expansion of a single EBV-infected progenitor cell [46,47].

FAMILIAL AGGREGATION

Even though majority of lung cancer is proved to be caused by tobacco use, only10–15% of all smokers develops lung cancer. Individual susceptibility is possibly due to genetic variations that affect carcinogen activation or catabolism [41].

Few studies suggest that the pattern is consistent with Mendelian inheritance of a rare major gene, particularly when there is tumor occurrence in young age .Lung cancer has been reported in some patients with Li–Fraumeni syndrome, which is due to inherited mutations in p53[42]. Lesser known cancer susceptibility syndromes have been linked to cause lung cancer.

GENETICS

Susceptible genes that are associated with carcinogen metabolism and DNA repair are CYP1A1, GSTM1[43]. CYP1A1 is a P450 enzyme involved in metabolizing several potential carcinogens, and two specific polymorphisms of the CYP1A1 gene have been linked to significantly increased lung cancer risk [44].

The relative deficiency of GSTM1, an enzyme involved in detoxifying metabolites of constituents in cigarette smoke, has been associated with lung cancer risk. Inherited variability in DNA repair mechanism also contributes to

inherited susceptibility to lung cancer by allowing the accumulation of genetic changes.

CANCER BIOLOGY:

The biology of lung cancer differs between smokers and non -smokers. Mostly all lung cancers occurring in non-smokers are adenocarcinomas, frequently well-differentiated tumors with lepidic features, and they have an improved survival compared to smokers[45].

Recent data have shown that lung cancers in never-smokers are much more responsive to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs),due to a greater likelihood of somatic mutation in the kinase domain of the EGFR[46]. Whereas K-ras mutations, predicts a poor response to therapy and shorter survival, are found frequently in smokers, and less so in non-smokers.

SYMPTOMS RELATED TO A CENTRAL TUMOR

Cough, Hemoptysis, Wheeze, Dyspnea and Pneumonia

SYMPTOMS RELATED TO A PERIPHERAL TUMOR

Pain,Cough, Dyspnea[51]

SYMPTOMS RELATED TO REGIONAL SPREAD OF TUMOR IN THE THORAX

Tracheal obstruction, Esophageal compression with dysphagia

Bronchopulmonary fistula, Recurrent laryngeal nerve paralysis with hoarseness Sympathetic nerve paralysis with Horner's syndrome [52], Pancoast syndrome, Superior vena cava syndrome due to vascular obstruction Pericardial and cardiac extension with tamponade, arrhythmia, or cardiac failure Lymphatic obstruction with pleural effusion [53].

HISTIOGENESIS:

Bronchial epithelium is the site of origin of lung carcinomas, causing 95% of primary lung tumors. Amongst the Non-small cell lung cancer, Squamous cell carcinoma was the leading histological type in terms of frequency. At present, it ranks second to adenocarcinoma[54,55] but remains a major cause of morbidity and mortality. Small cell and Squamous cell carcinomas are centrally located, near the hilum and prone to present with hemoptysis and early symptoms related to bronchial obstruction.

As a result of their central location, squamous cell carcinomas [56] are also accessible to the bronchoscope and are diagnosed early by means of biopsy and/or exfoliative and sputum cytology. Often achieving a large size and due to extensive necrosis, squamous cell carcinomas frequently cavitate, presenting as abscess-like masses, radiographically.

Contrasting small cell and squamous cell lung carcinomas which are centrally located, most adenocarcinomas are peripheral, subpleural in location [58]. The few that are central may be visualized endoscopically as endobronchial polyoid masses. Radiographically, adenocarcinomas usually present as a

nodular subpleural mass of various sizes and are often diagnosed as solitary pulmonary nodule. Large cell carcinoma represents about 10% of all lung cancers. Microscopically, its main feature is the high grade of malignancy that lacks squamous or glandular features.

Small cell carcinomas occur in middle age and older individuals with an average age of 50 to 55 years [59]. Cough, hemoptysis and chest pain are most common presenting symptoms. On chest radiography and computed tomography (CT), small cell carcinomas appear as hilar or perihilar masses, often in continous with mediastinal lymphadenopathy and lobar collapse. A small percent of tumors are peripherally located and in these instances the radiographic presentation will be that of a solitary lung nodule [60,61].

PATHOGENESIS:

Stepwise accumulation of genetic abnormalities results in transformation from benign bronchial epithelium to neoplastic tissue [56]. Large areas of respiratory mucosa are mutated after exposure to carcinogens (eg. smoking) → provides fertile ground for more cellular mutations that lead to cancer [57].

Genetic changes may include; 3p tumour suppressor deletion, p53 mutations, p16 mutations (NSCLC) and RB mutations (SCLC).

Investigations for lung cancer:

The gold standard test for diagnosis of lung malignancies is by histopathological examination of lung tissue specimens.

The diagnostic modalities used are

- ❖ Cytology of sputum specimen
- ❖ Thoracocentesis
- ❖ Flexible bronchoscopy(FOB)
- ❖ Transthoracic needle aspiration
- ❖ Thoracotomy
- ❖ Video assisted thoracoscopy
- ❖ Excision biopsy of nodes

For complete diagnosis, appropriate test procedure, with histopathological examination and staging is done.

WHO classification of malignant epithelial lung tumors (ANNEXURE-III)

- Small cell carcinoma
- Squamous cell carcinoma
    - Variants: papillary, clear cell, small cell, basaloid
- Adenocarcinoma
    - Variants: acinar, papillary bronchioloalveolar, solid adenocarcinoma with mucin, adenocarcinoma with mixed subtypes, fetal, mucinous, signet ring, clear cell.

- Large cell carcinoma
    - Variants: large cell neuroendocrine carcinoma, basaloid, lymphoepithelioma-like,
- clear cell, rhabdoid phenotype
- Adenosquamous carcinoma
- Sarcomatoid carcinoma
    - Variants: pleomorphic, spindle cell, giant cell, carcinosarcoma, pulmonary blastoma
- Carcinoid tumors
    - Variants: typical, atypical
- Carcinomas of the salivary gland type (rare)
    - Variants: mucoepidermoid, adenoid cystic, epithelial myoepithelial

PRECURSORS OF LUNG MALIGNANCY:

The World Health Organization (WHO) classification of lung cancer recognizes three preinvasive diseases that are thought to be precursors of malignant lung tumors. These are squamous dysplasia/carcinoma in situ (SD/CIS), atypical adenomatous hyperplasia (AAH), and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH).

BASAL CELL HYPERPLASIA

Basal cell hyperplasia (BCH), also known as reserve cell hyperplasia, is defined by the presence of three or more layers of basal cells in otherwise

normal respiratory epithelium. With a strict definition of BCH, these cells do not necessarily show evidence of keratinization or intercellular bridge formation and atypia is lacking.

GOBLET CELL HYPERPLASIA:

The patients with chronic bronchitis and asthma frequently show excess numbers of mucus-secreting goblet cells in the respiratory epithelium. There may be ciliated cells admixed with the goblet cells or short runs of only goblet cells may be present sometimes with a slightly papillary or tufted appearance. The cells lack nuclear atypia.

DIFFUSE IDIOPATHIC PULMONARY NEUROENDOCRINE CELL HYPERPLASIA (DIPNECH) (ANNEXURE-III):

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is an exceedingly rare lesion. It is included in the WHO classification of pulmonary preinvasive lesions because some patients with this disease develop one or more peripheral-type spindle cell carcinoid tumors. The characteristic presentation is of a slowly progressive disease by a typical unproductive cough and shortness of breath. Patients have age range between 40 and 60 years old, and there is a predominance of females.

Microscopically, Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is characterized by the widespread proliferation of Pulmonary neuroendocrine cells (PNC) in the form of increased numbers of single cells, small groups, and linear intraepithelial proliferations .

Larger nodules of cells may protrude into the bronchial or bronchiolar lumen, but remain covered by respiratory epithelial cells and are contained by the basement membrane. There may be bronchiolar fibrosis associated with these more pronounced collections of cells, and the fibrosis or the nodules of pulmonary neuroendocrine cells themselves may cause bronchiolar obstruction

SQUAMOUS METAPLASIA

In squamous metaplasia the full thickness of pseudo stratified respiratory epithelium is replaced by a population of squamous cells showing intercellular bridges in an intermediate zone lying above the basal cell layer and below a superficial zone of cell maturation, flattening, and keratinization. More frequently, keratinization is minimal and in squamous metaplasia the cells are not atypical.

While there is a strong association between squamous metaplasia and cigarette smoking, other factors such as exposure to irradiation, air pollution, smoking marijuana, vitamin A deficiency, and chronic lung diseases such as bronchiectasis, tuberculosis, and pneumoconiosis may also be responsible. Squamous metaplasia may be seen in airways draining chronic suppurative lesions and around the site of tracheostomy or other points of bronchial trauma, and may be found overlying a typical carcinoid or a variety of benign bronchial tumors .

Basal cell hyperplasia, loss of ciliated cells, and squamous metaplasia may be seen in association with pneumonia, but atypia is not seen. Chronic irritation is the factor that induces this adaptive change to an epithelium better able to deal with the prevailing environment. In vitamin A deficiency both hyperplasia and squamous metaplasia of the tracheobronchial epithelium occur. Since many of the dysplasias and carcinomas-in-situ that occur in the human airway have squamous features, squamous metaplasia is often assumed to be the precursor of squamous dysplasia and CIS.

SQUAMOUS DYSPLASIA / CARCINOMA-IN-SITU

This type of lesions, occurs in tracheobronchial, and to a lesser extent in bronchiolar epithelium, is the well known of the preinvasive lung lesions. It is likely that most bronchogenic squamouscell carcinomas, but also many small cell lung cancers and possibly other tumor types arising in the central airways, develop from such alterations in the airway epithelium.

Grading of dysplasia in tracheobronchial epithelium by WHO classification has four categories: mild, moderate, severe dysplasia, and carcinoma in situ. Although four categories are described, it is recognized that these changes represent a biologic continuum, and the divisions are artificial.

Epithelial thickness, cell size, changes in maturation and orientation, and nuclear features assessed, in some cases cell maturation is complete, resulting in keratinization, while in others a more basaloid phenotype is retained.

MILD DYSPLASIA

The key feature in diagnosis is expansion of the basal zone of cells into, but not beyond, the lower third of the epithelium. Atypia and pleomorphism are minimal, but the crowded cells in the lower third have vertically orientated nuclei with few irregular features. Mitoses are absent or very rare.

MODERATE DYSPLASIA

In moderate dysplasia the crowded population of basal cells with vertically orientated nuclei extends into, but not above, the middle third of epithelium. Epithelial thickness is increased, cell size is increased, and nuclear contours are irregular. Mitotic figures may be present anywhere in the lower third of the epithelium.

SEVERE DYSPLASIA& CARCINOMA IN SITU:

It is characterized by a marked increase in cell size, pleomorphism, and nuclear variability, nucleoli. The crowded basal zone of cells clearly extending into the upper third of the epithelium both mitoses and vertically orientated nuclei may be found in the lower two thirds of the epithelium There remains evidence of flattening of epithelial cells on the surface.

The main feature in the recognition of Carcinoma in Situ is a completely haphazard orientation of markedly enlarged and pleomorphic cells. There is usually no evidence of cell maturation such that if the epithelium were inverted it would look the same. Mitotic figures may be found at any layer. As

atypia develops in this squamous-type epithelium, the basement membrane also gets thickened.

MOLECULAR EVENTS:

Developments in immunohistochemistry, molecular biology, and genetics have led to considerable advances in our knowledge of the molecular events. That mainly occurs during the progression of preinvasive disease in the bronchial epithelium.

A large number of studies have shown that there is progression in the patterns of protein expression and genetic alterations in step with the morphologic progression of disease, increasingly altered genotype determine the phenotype.

ALTERED CELL PROLIFERATION:

The evidence of the earliest precursors, as well as Squamous Dysplasia /Carcinoma in Situ, demonstrates various degrees of hyper proliferation. The presence of increased numbers of often abnormally located mitotic figures is part of the definition of Squamous Dysplasia /Carcinoma in Situ. Using anti–proliferating cell nuclear antigen (PCNA)antibodies, Hirano et al. demonstrated that, while the proliferating compartment of normal mucosa was confined to the lowest 25% of the respiratory epithelial cells.

Proliferating respiratory epithelial cells expanded to 35% to 40% of the epithelium in low- and high-grade dysplasia, while in invasive disease, 85% to

90% of cells were cycling. Others have found similar results, with PCNA-positive nuclei co-located to the expanded basal layer found in bronchial dysplasia or by using anti-MIB1 antibodies.

NEOVASCULARISATION:

Studies show significant rise in sub epithelial vessel count when comparing hyperplasia/metaplasia, moderate squamous dysplasia, and Carcinoma In Situ. Similar associations between disease progression and micro vascular density were reported by Fontani et al., They also found an increasing expression of vascular endothelial growth factor (VEGF) and p53 protein, though between dysplasia and CIS.

p63:

Massion et al found that the p63 gene at 3q27 is amplified in most squamous cell carcinomas .The p63 protein is a member of the p53 family, a p53 homologue that transactivates *P53* genes and induces apoptosis in cells expressing one of the six splice variants.

Increase in gene copy number was also found in severe dysplasia and CIS, but not in lower grade lesions, and this correlated with an increase in stainable p63 protein in the expanded basal cell layers.

The Fragile Histidine Triad (FHIT):

The FHIT gene located at 3p14.2 spans the FRA3Bcommon fragile site and is a putative TSG with possible functions related to apoptosis and control of cell proliferation.

It is frequently lost in many human tumors including lung cancer.

Loss of immunohistochemically detectable FHIT protein was found in 93% of preinvasive bronchial lesions overall (in 60% of moderate dysplasias and all severe dysplasia and CIS), and may be an important and early change in squamous cell carcinogenesis, a suggestion supported by Geradts et al.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR):

The epidermal growth factor receptor (EGFR) ERBB1, one of the ERBB family of transmembrane receptor tyrosine kinases, has epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) as ligands, and regulates epithelial cell proliferation and differentiation[(106)].

EGFR is frequently over expressed in NSCLC, particularly adenocarcinoma, as opposed to small cell lung cancer (SCLC), and has been studied in bronchial preinvasive lesions.

PHOSPHORYLATED AKT (PROTEIN KINASE B):

The serine/threonine kinase Akt is a downstream effector of the phosphatidylinositol 3-kinase (PI3K) pathway, the activation of which cause

malignant transformation in animal models of human cancer. AKT may be activated in respiratory epithelial cells by components of tobacco smoke.

Immunohistochemical detection of activated p-AKT Ser473 was found in 27% of normal bronchial epithelia, 44% of hyperplasias, and 88% of dysplasias, but in only 33% of invasive NSCLCs. Expression in invasive disease was not related to tumor histology, and the authors concluded that p-AKT activation is an early event in bronchial carcinogenesis.

SQUAMOUS CELL CARCINOMA:

Most commonly SCCs arise in main bronchi, lobar, segmental, or sub segmental bronchi, about one third arise from small peripheral alveoli. Usually, SCCs are smaller than other lung carcinomas since obstructive symptoms manifest early in the clinical presentation [58].

Grossly, tumors are tan white or gray with hemorrhage and necrosis of variable degree. Large tumors are prone to central cavitation, but small tumors also cavitate. Lesions can be firm or soft depending on the amount of stromal desmoplasia, keratin production, and necrosis. Central tumors form intraluminal polypoid masses and mucosal thickening, and often infiltrate through the bronchial wall into peribronchial tissue, lung, hilar and mediastinal lymph nodes, mediastinal structures.

These are malignant epithelial tumors arising from a progressive dysplasia of metaplastic squamous epithelium. Most Squamous cell carcinomas probably arise from metaplastic basal cells, columnar goblet cells may also

develop into carcinoma through the metaplasia-dysplasia-carcinoma sequence. Keratinpearl, keratinization or intercellular bridges are the diagnostic morphologic features of Squamous cell carcinoma (SCC).

Many morphologic variants has been described, the current WHO classification recognizes papillary, clear cell, small cell, and basaloid. In addition, Squamous cell carcinoma may be combinedwith adenocarcinoma, SCLC, and other malignancies, even in endobronchial locations.

Cytological diagnosis of squamous cell carcinoma from bronchial washings, sputum samples depends on the identification of dysplastic squamous cells. Dysplasia is cytologically graded based on nuclear morphology, keratinisation and amount of cytoplasm, nuclear/cytoplasmic (N/C) ratios; the higher the ratio, the less differentiated the cell is and higher the grade of dysplasia. Cells are present isolated, in aggregates, and in tissue fragments.

With immunohistochemical marker, there is strong reactivity for low & high molecular weight keratins, p63,p40 ,involucrin[(76)]. Squamous cell carcinoma is also positive for vimentin EMA, S100, desmocollin-3.

ATYPICAL ADENOMATOUS HYPERPLASIA (AAH):

Adenocarcinoma arising from pulmonary scars or fibrosis, the lesion is now called atypical adenomatous hyperplasia (AAH) as recognized in the WHO classification as a putative precursorof invasive adenocarcinoma. The majority of Atypical Adenomatous hyperplasia lesions are incidental

microscopic findings, occasional lesions may be detected on gross examination of the cut surface of the lung.

Lesions are small, a few millimeters in diameter, and may be visible as discrete, or ill-defined, grayish, tan, or yellowish foci. Occasionally the lesion may be large and clear enough to allow appreciation of the alveolar architecture by the presence of a stippled pattern of depressions or holes on its cut surface. The Atypical adenomatous hyperplasia lesions are most often found close to the pleura and in the upper lobes.

Microscopically, Atypical adenomatous hyperplasia is defined as a localized proliferation of mild to moderately atypical cells lining involved alveoli and sometimes respiratory bronchioles, resulting in focal lesions in peripheral lung, usually less than 5 mm in diameter and generally in the absence of underlying interstitial inflammation and fibrosis in the latest WHO classification.

ADENOCARCINOMA:

Mostly these tumors are located peripherally gives rise to clinical symptoms on reaching larger size and invading adjacent structures [(58)]. Grossly, adenocarcinoma more often involves the upper lobes and typically presents as a subpleural mass or nodule with retraction of the pleura. Friedrich (1939), Rossle (1943), and later Spencer (1985) has described the association of peripheral lung cancers, mainly adenocarcinomas, with sub pleural scars.

Alveolar interstitial fibrosis is almost always present at the margins of scarring in the lung, regardless of the cause, and is associated with reparative hyperplasia of bronchiolar and alveolar epithelial cells, a change that was found to precede neoplasia in some cases.

The occurrence of lepidic carcinoma in patients with scarring due to cystic lung diseases, tuberculosis, or autoimmune disorder (progressive systemic sclerosis) of the lung are cited as examples of neoplasia arising in the alveolar septal fibrosis that associated epithelial hyperplasia .

Shimosato (1982) explained that atypical adenomatous hyperplasia (AAH) of bronchoalveolar epithelium may arise in otherwise normal lung tissue as a precursor of peripheral adenocarcinomas of the lung. Pneumocytes type II as well as Clara cells may participate in the pathogenesis of lepidic carcinoma that has been confirmed by electron microscopy, and immunohistochemistry.

The tumor is characterized by glandular differentiation, mucin production by the tumor cells. These lesions are usually more peripherally located and tend to be smaller. Cavitation is extremely unusual.

Adenocarcinoma present as single peripheral nodule, multiple nodules and diffuse pneumonic like infiltrate. Histopathologically the mucinous type is formed by well differentiated mucin containing columnar cells that line respiratory spaces without invading the stroma. The sharp separation is often found between the neoplastic and normal cells, a useful diagnostic feature. The

cells of non mucinous type of adenocarcinoma are cuboidal rather than columnar and have bright eosinophilic cytoplasm. Nuclear atypia and prominent nucleoli are greater than in mucinous variant.

Grading of adenocarcinoma into well, moderately, and poorly differentiated tumors depends on the degree and extent of glandular differentiation and as well as the degree of cytologic atypia.

Variants: Acinar, papillary bronchioloalveolar, solid adenocarcinoma with mucin, adenocarcinoma with mixed subtypes, fetal, mucinous, signet ring, clear cell.

Cytologically, adenocarcinoma and its precursor tends to exfoliate both as single cells and cell groups. The nuclei are typically round to oval and uniform in size, with finely granular or powdery chromatin and small, indistinct nucleoli. In few cases, however, show prominent nucleoli. Nuclear folds are usually present and in some cases nuclear pseudo inclusions are seen. The cytoplasm is in moderate to abundant with homogeneous, granular, finely vacuolated, or distended by single or multiple large vacuoles.

Immunohistochemically, they are positive for low molecular weight keratin, CEA, EMA, TTF-1 & members of MUC family [(82)]. Associated with genetic features like TP53 alteration, p16/CDKN2A inactivation, disruption of RB pathway, loss of 3p, KRAS, EGFR and C-MET mutation [(83,85)].

SMALL CELL CARCINOMA:

Small cell carcinoma comprises 10–15% of all lung cancers. Most of the patients are males, their median age is 55 to 60 years, and 90 % or more are smokers [59,60].The significance of its distinction from other types of lung carcinoma due to its clinical behavior, systemic nature, and responsiveness to chemotherapy. In 1926, Barnard became the first to classify it as bronchogenic carcinoma, and, after the elaborate work by Azzopardi, it became an distinct entity [61].

Small cell carcinoma is characteristically a lesion of the central portions of the lung, but on rare occasions it is found in a peripheral location. Bronchoscopic biopsy is often positive, even if no gross lesions are seen.

Grossly, the tumor is grey white to tan, soft, friable, and extensively necrotic. While located in a large bronchus (the most common situation), it may involve it in a circumferential fashion and spread widely beneath the normal mucosa. The bronchus may be totally occluded in the late stages, but pure or predominant endobronchial involvement is highly unusual.

Histologically small-cell carcinoma is characterized by diffuse sheets of small cells ; these cells have hyperchromatic nuclei, indistinct nucleoli, scant cytoplasm and indistinct cell border. The pattern of growth is generally solid, other patterns are streams and ribbons, rosettes and pseudorosettes, or tubules and ductules [62].

Small cell carcinoma is characterized by intense basophilic staining of vessel wall due to encrustation caused by DNA from necrotic tumor cells called the Azzopardi effect, is frequently present. IHC, they are positive for Bcl2 and mutation of p63 and RB tumor suppressor genes are commonly present.

Cytologically, the neoplastic cells of SCLC are arranged in loose, irregular cell aggregates, or syncytial tissue fragments. Individual single cells, often in a necrotic background, are also present. The neoplastic cells are small to intermediate in size (less than three times the size of resting lymphocyte). The shape of individual cells varies from round to oval to spindle shaped.

The cells have scant cytoplasm, and therefore the nuclear cytoplasmic N/C ratio is extremely high. The Mitotic count is high, even though well preserved mitotic figures may be difficult to identify in a cytology specimen. The chromatin pattern is finely granular in most cells, and nucleoli are usually small or absent.

LARGE CELL CARCINOMA:

These tumors are characterized histologically by sheets of large tumor cells with round to oval nuclei, prominent nucleoli, increased mitotic activity, and marked cellular atypia [(66)]. Another distinctive feature in some cases is the presence of abundant inflammatory infiltrate in the stroma, admixed with the anaplastic tumor cells.

Variants: large cell neuroendocrine carcinoma, basaloid [(67)], lymphoepithelioma-like, clear cell, rhabdoid phenotype[(68).]

Like adenocarcinoma, large cell carcinoma (LCC) is often a peripheral neoplasm. It tends to be large, so an endobronchial component is often present. Cytologic samples of large cell carcinoma show highly pleomorphic population of loosely aggregated or individual cells. There is no cytologic feature suggesting differentiation.

The cells have a high nuclear cytoplasmic N/C ratio and the cytoplasm is dense. The nuclear contours are irregular and nuclear chromatin is coarse. Prominent nucleoli are readily identified.

ADENOSQUAMOUS CARCINOMA:

Adenosquamous carcinoma, both adeno and squamous cell carcinomatous component present in the same tumor. This accounts for about 3- 5% of lung cancers. It arises both in the major bronchi and periphery of the lung. Most of the cases are situated peripherally and often are associated with a scar, depicting a closer relationship with adenocarcinoma.

The term adenosquamous carcinoma is used for lung tumors in which indisputable evidence of squamous and glandular differentiation found in the same tumor in a roughly equal proportion [(69)]. Squamous cell carcinomas having occasional mucin-producing cells or adenocarcinomas with minute foci of squamous differentiation are classified in relation to their predominant component.

SARCOMATOID CARCINOMA:

The recent World Health Organization (WHO) classification of lung tumors classifies sarcomatoid carcinomas as a group of poorly differentiated, non–small cell lung carcinomas that contain a component of sarcoma or sarcoma like (spindle or giant cell) differentiation. Five subgroups are recently recognized: pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma[69,70].

Grossly, these tumors can be intraparenchymal or intrabronchial polypoid masse. Histologically, the epithelial elements are usually of squamous type, also have a glandular appearance. The sarcoma-like component may be of fibrosarcoma- or MFH-like appearance or resemble chondrosarcoma, osteosarcoma, rhabdomyosarcoma, or angiosarcoma.

On rare occasions the tumor has rhabdoid features. Osteoclast-like giant cells can be present. The distinction between the carcinomatous and the sarcoma-like components can be vague or distinct. Immunohistochemically, they are positive for pankeratin, EMA and p63.

Cytologically, adequate samples are quite cellular, with the tumor cells manifesting as single cells, clusters, or syncytial fragments. The tumor cells are large with large nuclei and nucleoli. The N/C ratio may be variable, and the shapes of the cells range from round to polygonal to spindle shaped.

The category of sarcomatoid carcinoma is further subdivided into pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma,

carcinosarcoma, and pulmonary blastoma. Definite cytologic diagnosis of these tumors is extremely difficult since marked intratumoral heterogenecity is noted in many cases.

CARCINOID TUMORS:

Carcinoid tumors are regarded as tumors of low-grade malignancy. Incidence is about 1% to 2% of all lung tumors. These tumors relatively common in persons younger than it is usual for lung cancers, and the male-to-female ratio is about 1:1. The tumor is considered to arise from Kulchitsky cells [63,64], it belongs to the dispersed endocrine cells system.

Most of these tumors arise in the main to segmental bronchi, but peripheral origin is occasionally seen. Grossly, the tumor is polypoidal mass, endobronchial in the major bronchi, solid and nodular in the periphery of the lung. The tumor is circumscribed, having a smooth, sometimes lobulated appearance, glistening cut surface. Necrotic foci are mostly not seen.

Histologically, tumor is composed of nests, trabeculae, and mosaic patterns of medium-sized polygonal cells with oval to round uniform, finely granular nuclei, and pale eosinophilic to clear cytoplasm. Rosettes and small acinar structures with or without mucin may be present. Mitotic figures are rare [65]. Peripherally located tumors may be composed of spindle-shaped cells. The stroma is relatively more vascular and amyloid deposits may be seen.

The recent WHO classification defines typical carcinoid as a tumor with fewer than2 mitoses per 10 high-power fields and lack of necrosis. There may be cytologic atypia, increased cellularity, and lymphatic invasion.

The atypical carcinoid is defined as a tumor with 2 to 10 mitoses per 10 high-power fields and/or with foci of necrosis, which are usually punctate. Cellularity, irregular architecture, cytologic atypia , prominent nucleoli and lymphatic invasion may be seen, but the most important criteria is the mitotic count.

Cytologically, the tumor cells present as clusters or dispersed individual cells. The clusters may aggregate in a nested, acinar, sheet-like, or trabecular pattern. On occasion, gland-like or papillary structures, may mimick adenocarcinoma.

The shapes of the individual cells range from round to oval to spindle. The cells are uniform with a moderate amount of finely granular cytoplasm, which may be amphophilic or eosinophilic. Nuclei are round to oval with smooth nuclear membranes. Nucleus may be eccentrically placed, giving a plasmacytoid appearance.

OTHER RARE TYPES OF LUNG CANCERS:

There are many rare types of lung cancers like adenoid cystic carcinoma, mucoepidermoid carcinoma and epithelial-myoepithelial carcinoma [72,73].

ADENOID CYSTIC CARCINOMA:

Patients age range is from 16 to 80 years, with an average in most series of 45 to 48 years. Men and women are about equally affected, and tobacco exposure is not a risk factor. Symptoms, usually resulting from partial airway obstruction, include wheezing, progressive shortness of breath, and hemoptysis.

Grossly, the tumor masses are rubbery to quite firm and pink-tan to gray-white. The masses are always infiltrative. Histologically, these tumors may infiltrate bronchi in a radial fashion and encase blood vessels. They appear identical to those in the salivary gland tissue, with compact nuclei, a relatively high nuclear/cytoplasmic ratio, cysts rather evenly contoured but of varying caliber within larger tubules of tumor, and the stroma is often hyalinized. Mitoses and necrosis are less common.

MUCOEPIDERMOID TUMORS:

Mucoepidermoid tumors are microscopically characterized by a mixture of mucus-secreting cells, squamous cells, and intermediate type of cells, and represent the second most common tumors of bronchial gland tumors, after adenoid cystic carcinomas. Grossly, mucoepidermoid tumors, especially the lower grade ones, are usually polypoid in the bronchi, and the higher-grade lesions are less polypoid than the low-grade lesions. They do not extend in as linear a fashion along the wall of the airways as do adenoid cystic carcinomas, discussed later in this chapter.

Microscopically, the low-grade lesions show significant heritage to bronchial glands, with abundant mucinous cysts staining with acidic, weakly acidic, and neutral mucosubstance stains, as well as goblet cells. The cyst like spaces or goblet cells may be predominant within the tumor. The mucoepidermoid nature is determined by more solid collections of nonkeratinizing squamoid or transitional cells or bland pale cells adjacent to these cysts, sometimes arranged as small nests or larger sheets.

Some of these cells appear intermediate between glandular and true squamous cells. Focal keratinization may or may not be present but is generally rare. Rare mitoses are present in the low-grade lesions, and some of these tumors overlap with those described as mucous gland adenomas. The high-grade lesions have an abundance of solid sheets of intermediate cells.

PLEOMORPHIC ADENOMA:

Endobronchial or tracheal pleomorphic adenomas are usually soft to rubbery, polypoid nodular masses ranging from 1.5 to 15 cm in diameter, covered by intact respiratory epithelium. The cut surfaces are gray-white and shiny due to their myxoid content. The gross appearance of parenchymal tumors, is not well described but mimics the tumors in the salivary glands of the head and neck.

Microscopically these tumors appear in the assorted patterns seen in the mixed tumors of the salivary glands. They have abundant glandular epithelial and stromal components, with varying degrees of differentiation. Typically, a

gradual merging pattern between the epithelial and stromal components is seen in most tumors. The epithelial cells are usually arranged in tubules or cell clusters, and have an angulated form and sometimes line flattened small ducts. Mitoses and necrosis are infrequent in pleomorphic adenoma.

METASTATIC CARCINOMA:

Metastatic tumors to the lungs constitute up to 10-20% of lesions diagnosed by biopsy and cytology in some studies. Complete knowledge of the clinical history must be available, together with earlier cytological and histopathological diagnosis for review and comparison with current material. Metastatic tumors, mainly renal cell and colonic carcinomas, may mimic primary tumors clinically either by growing as an endobronchial lesion or because of a lepidic growth pattern.

Cytologically aggregates of tumor cells seen in clean background. Cell blocks, special stains, immunohistochemistry helpful. Some metastases are recognizable morphologically. For example, metastatic well differentiated colonic carcinoma often presents with palisading, elongated nuclei in aggregates and in a background of confluent necrosis.

LYMPHOMA, LEUKAEMIA AND OTHER DISORDERS:

Non-Hodgkin’s lymphomas of extrapulmonary origin and of all histological subtypes quite frequently affect the lung during the course of the disease. Hodgkin’s lymphoma is rarely seen as primary lung lesions, but the lung is a common site of relapse, particularly for nodular sclerosing disease.

Tumor deposits are usually nodular and may cavitate or produce endobronchial lesions.

Diagnosis of recurrent tumor is often possible cytologically but, definitive diagnosis of primary lymphoma generally requires open biopsy. However, sufficient material for immunophenotyping is usually considered necessary for definitive diagnosis.

Large cell lymphomas are easier to diagnose than small / mixed. Loosely aggregated lymphoid cells, with intact cytoplasm, vesicular nuclei, no moulding, and nucleoli visible. Subtyping is possible in BAL and FNA material. Large cell lymphomas are most easily diagnosed, small or mixed cell lesions are diagnostically difficult.

RECOMMENDATIONS BY IASLC/ATS/ERS NEW MULTIDISCIPLINARY INTERNATIONAL CLASSIFICATION, FOR SMALL BIOPSY AND CYTOLOGY SPECIMENS:

- For cytology as well as small biopsy specimens, if a clear differentiation can be done, which satisfies the standard morphologic criteria, further specific typing of NSCLC into squamous cell carcinomas and adenocarcinomas can be done with morphology alone.
- The term NSCLC - NOS must be used as infrequently as possible and it should only be used if the diagnosis cannot be made out by morphology and /or by special staining / IHC.

- When small biopsy / cytology specimen is used in addition with special stains for diagnosis, it should be clearly noted whether the diagnosis is achieved with only light microscopy or in combination with special stains.
- The term non-squamous cell carcinoma which is used by clinicians should not be used by pathologists while reporting. Pathologists should report NSCLC only as ADC, SQCC and NSCLC – NOS.
- The tissue specimens received by pathologists should be used judiciously and preserved to the maximum, as more tissues will be needed for further molecular studies.
- In small biopsies / cytology specimens, if any invasive pattern is found in adenocarcinoma, it is to be reported as a lepidic growth pattern. The term minimally invasive ADC and ADC- in situ should not be used. The term large cell carcinoma, should be used only in resected specimens as thorough sampling of tumor is not possible in small biopsy/cytology specimens
- If the tumor shows sarcomatoid features characterized by malignant giant cells or spindle cells with nucleus showing pleomorphism, it should be classified according to guidelines above as NSCLC favouring ADC or NSCLC favouring SCC based on features of glandular pattern or squamous features respectively. When these features are absent it is to be reported as NSCLC -NOS with a word about sarcomatoid features.

- Only if the tumor shows neuroendocrine morphology, neuroendocrine IHC markers are performed.
- Further classification of NSCLC- NOS is possible with the use of IHC, into NSCLC favouring ADC and NSCLC favouring SCC.
- It is advised to use minimal stains for further sub classification of NSCLC-NOS.
- It is recommended to use only one marker for adenocarcinoma or one marker for squamous cell Carcinoma.
- Currently, the single best marker for diagnosing adenocarcinoma is TTF-1.Staining with diastase - periodic acid schiff, alcian blue/ PAS stains or mucicarmine also play a role in diagnosing adenocarcinoma.
- The specific marker for diagnosing SCC is Polyclonal p40 rather than the monoclonal p63. p40 is likely to surpasses p63 as a best IHC marker in diagnosing squamous cell carcinoma. In NSCLC -NOS, the cases which shows TTF-1 positive and /or mucin positive, but p40 and p63 negative are termed as NSCLC favouring adenocarcinoma. Similarly those cases with p40 and/or p63 positive but TTF-1 and mucin stain negative are termed as NSCLC favouring SCC with comment on whether special stains are used to arrive at diagnosis.
- In case, one population of tumor cells show TTF-1 reactivity and another population of tumor cells show positive for squamous cell markers, possibility of adenosquamous carcinoma should be considered.

- But if TTF-1 as well as p40 if negative and fails to show any squamous or glandular morphology, the diagnosis still remains as NSCLC-NOS.

STAGING OF LUNG CARCINOMAS:

Primary tumor (T):

TX Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy

T0 - No evidence of primary tumor

Tis - Carcinoma *in situ*

T1 - Tumor 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus[87], (i.e., not in the mainbronchus)

T2 - Tumor with any of the following features of size or extent: More than 3 cm in greatest dimension .Involves main bronchus, 2 cm or more distal to the carina.

Invades the visceral pleura. Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung[88].

T3 Tumor of any size that directly invades any of the following:

Chest wall (including superior sulcus tumors), diaphragm, mediastinal pleura, parietal pericardium; or tumor in the main bronchus less than 2 cm distal to the carina[89], but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung.

T4- Tumor of any size that invades any of the following:

Mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; or separate tumor nodules in the same lobe; or tumor with malignant pleural effusion.

REGIONAL LYMPH NODES (N):

- NX Regional lymph nodes cannot be assessed
- N0 No regional lymph node metastasis
- N1- Metastasis to ipsilateral peribronchial and/or ipsilateral hilar lymph nodes, and intrapulmonary nodes including involvement by direct extension of the primary tumor
- N2- Metastasis to ipsilateral mediastinal and/or subcarinal lymph nodes(s)
- N3 Metastasis to contralateral mediastinal, contralateral hilar,ipsilateral or contralateral scalene, or supraclavicular lymph nodes(s)

DISTANT METASTASIS (M):

- MX- Distant metastasis cannot be assessed
- M0- No distant metastasis
- M1- Distant metastasis present
- M1- includes separate tumor nodule(s) in a different lobe (ipsilateral or contralateral)

PROGNOSIS:

The long-term prognosis of lung carcinoma remains discouragingly poor, in long-term survival rates. In a large series of 1008 cases of lung carcinoma studied during mid 1950, the 5-year survival rate was 21.3% for the resected cases and 8% for the entire group. Similar results are obtained from various other groups during the last 30 years. PROGNOSTIC FACTORS: The prognosis of lung carcinomas has been related to a large number of factors:

AGE:

Patients of age younger than 40 years have a bad prognosis, probably due to advance stage of disease at presentation.

SEX:

Females are found to have a worse survival rate than male; due to the fact that they have a higher incidence of advanced lesions and of tumors with an adenocarcinomatous pattern.

LOCATION.

The tumors situated above superior pulmonary sulcus have a better prognosis than the others, the reported 5-year survival rates ranging between 20% and 34%.For squamous cell carcinomas, those located at the periphery are said to do better than those located centrally.

TUMOR SIZE :

Large the tumor size bad the prognosis than smaller neoplasms of the same histologic type. In case of adenocarcinomas with in situ and invasive components, the size of the latter is an independent predictor of survival(90).

PRESENCE OF A SCAR:

The peripheral adenocarcinomas and undifferentiated large cell carcinomas associated with a well-defined fibrotic area (scar) have a worse prognosis than tumors lacking this feature.

HISTOLOGICAL TYPE AND DEGREE OF DIFFERENTIATION:

Squamous cell carcinoma is the most curable form of lung cancer. In study of long-term survivors of lung tumors, nearly half of the cases were squamous cell carcinomas. The 5-year survival rate in patients undergoing resection for cure is about 40-50 % for well-differentiated tumors, 20% for moderately differentiated tumors, and 6 % for poorly differentiated tumors. In case of adenocarcinomas, the survival is about 25%, relatively not

influenced by the degree of differentiation. Among adenocarcinomas, those with a micropapillary adenocarcinoma pattern do worse than the others(95) . The prognosis of lepedic pattern is better than that of conventional adenocarcinoma .The localized form (usually having a nonmucinous histology) is curable in a high proportion of cases. Undifferentiated large cell carcinomas give a figure close to 15%.

In a series, undifferentiated large cell histology and presence of tumor giant cells in any histologic type were inadvertently associated with a worse outcome. Giant cell carcinoma is practically never curable.

Small cell carcinoma has been associated with a worse prognosis, the 5-year survival rate being less than 2% in most early series. A relative short-term improvement as a result of chemotherapy, but the long-term outlook remains bad.

RHABDOID FEATURES:

The presence of a rhabdoid component is a sign of very aggressive behavior as in other sites .

INFLAMMATORY REACTION:

The presence of a prominent lymphoplasmacytic reaction around the tumor is a favorable prognostic sign.

LYMPH NODE INVOLVEMENT:

The component of the staging system, one of the most important prognostic factor. It applies not only to the presence of nodal metastases but also to the anatomic level of these deposits. Also, the tumors associated with regional lymph nodes showing germinal center formation have a better prognosis than those in which the nodes have lymphocyte depletion or appear unstimulated.

STAGE:

TNM stage is regarded by most as the single most important prognostic parameter in lung carcinoma, as it is in many other tumors throughout the body. A direct relationship is evident between clinical stage and survival rates, particularly for non-small cell carcinoma.

BLOOD VESSEL INVASION: Poor prognosis, in association with lymph node metastases the adverse effect on survival is additive.

CHEST WALL INVASION: The tumors associated with invasion of the chest wall have prognosis not significantly different from non invasive,except the surgery related mortality which is relatively higher.

PLEURAL EFFUSION: It carries a poor prognostic indication regardless of histologic subtype or cytologic findings in the fluid. The presence of tumor cells in intraoperative pleural lavage is also additive to the poor prognosis.

TTF-1: Patients with non-small cell carcinomas exhibiting strong expression of this nuclear transcription factor predicts their better survival.

CD117: A subset of adenocarcinomas and squamous cell carcinomas of the lung immunoreactivity for CD117 has been found to correlate with a high degree of tumor proliferation and features of aggressivity.

DESMOGLEIN 3: Lack of desmoglein 3 in lung carcinomas (as well as atypical carcinoid tumors) at the immunohistochemical level are more aggressive than their counterparts featuring this desmosomal protein of the cadherin family

DNA PLOIDY: Multiple tumor site sampling is essential for determination of tumor DNA ploidy by flow cytometry which contributes to the prognostic assessment of lung carcinoma (95).

ONCOGENE EXPRESSION: Amplification of MYC genes in small cell carcinoma lines and expression of the RAS oncogene is enhanced in non-small cell carcinoma (NSCLC).

Studies have shown that increased expression of RAS P21 in non-small cell carcinoma, and of NMYC in small cell carcinoma, predict poor prognosis. An over expression of P53 and HER2/neu(96,97) are predictors of poor survival.

EPIDERMAL GROWTH FACTOR RECEPTOR:

EGFR is a trans-membrane glycoprotein belonging to erbB family of closely related receptor tyrosine kinases. It binds extracellular and intracellular tyrosine kinases and other regulatory domains. Hence the normal functioning EGFR undergoes conformational change and phosphorylation causing signal transduction and cell proliferation and inhibition of apoptosis (98).

The invention of EGFR receptor tyrosine kinase inhibitors gefinitb and erlotinib has redefined the treatment of lung cancer patients in advanced cases. A large phase III trial of nearly 1700 advanced lung cancer patients treated with gefitinib or placebo was conducted. It did not have survival benefit for all patients treated with lung cancer[(106)].

However, there was a significant survival advantage in non-smoking Asian women with adenocarcinoma[(99)] histological type. More recently afatinib, TKI drug approved for the treatment of patients with lung adenocarcinoma, have been shown to significantly extend progression-free and overall survival in patients those harbor activating EGFR mutations.

It has been assessed that EGFR is most likely expressed in solid growth pattern which suggest that there is a strong association between EGFR amplification (100) and aggressiveness. EGFR expression can be assessed by IHC, PCR and F1SH. Positive predictive values for each of them are 6.5-8.2%, 7-100% and 11-89% respectively.

Diagnosis by IHC Markers:

Paraffin blocks with 4 micron serial sections from surgical biopsy specimens or cytology cell blocks can be used for EGFR protein expression. Chromogen is used to identify positive result in diaminobenzidine. Negative internal control can be provided by normal epithelial and stroma. Tumor cell staining membrane is considered to be specific for the interpretation of the tumor.

It is estimated that IHC positive tumors show strong EGFR expression. Whereas IHC negative tumors have low or no expression. The introduction of targeted therapies has greatly redefined the treatment of advanced lung carcinomas. Two oral drugs which are used to inhibit EGFR receptors are Erlotinib & Gefitinib and they have been recently approved for use in advanced non small cell lung cancer. Thus clinical, morphological and molecular factors can predict the response rate to these drugs.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present prospective study conducted at Department of Pathology, Madurai medical college July 2015 to August 2017. Ethical clearance for the study was obtained from Ethical Committee of Madurai medical college, Madurai.

Total sample of 108 cases of suspected lung tumor small biopsy specimens were analyzed during the study period.

INCLUSION CRITERIA

Small biopsies of suspected lung tumors

EXCLUSION CRITERIA

Benign lesions

Other non neoplastic lesions

HISTOMORPHOLOGICAL EVALUATION:

Stained slides were evaluated under light microscopic examination. Tumors were classified and categorized broadly according to their pattern of differentiation. Tumors were graded based on cellularity, nuclear atypia and presence or absence of atypical mitotic figures, necrosis, lymph vascular invasion.

IMMUNOHISTOCHEMICAL EVALUATION

Paraffin blocks with 4 micron thick serial sections from small biopsy specimens are used for EGFR protein expression. Chromogen is used to identify positive result indiaminobenzidine. Negative internal control is by normal epithelial and stromal tissue.

IHC SCORING FOR EGFR:

Tumor cell staining membrane is considered to be specific for the interpretation of the tumor.

- Score 0 is given for tumors that has no staining of tumor cells,
- Score 1 weak membrane staining in more than 10% of tumor cells,
- Score 2 for moderate staining in more than 10% of tumor cells and
- Score 3 for tumor cells with strong intensity in more than 10% of tumor cells.

STATISTICAL ANALYSIS

Data obtained was entered into Microsoft excel spread sheet. The data was analyzed using ratios and percentage. Spearman‘s Rho and Pearman`s Coefficient correlation studies were done, p value was derived to determine the statistical significance level of the study. Observations and results were compared with other studies and inferences drawn.

OBSERVATION AND RESULTS

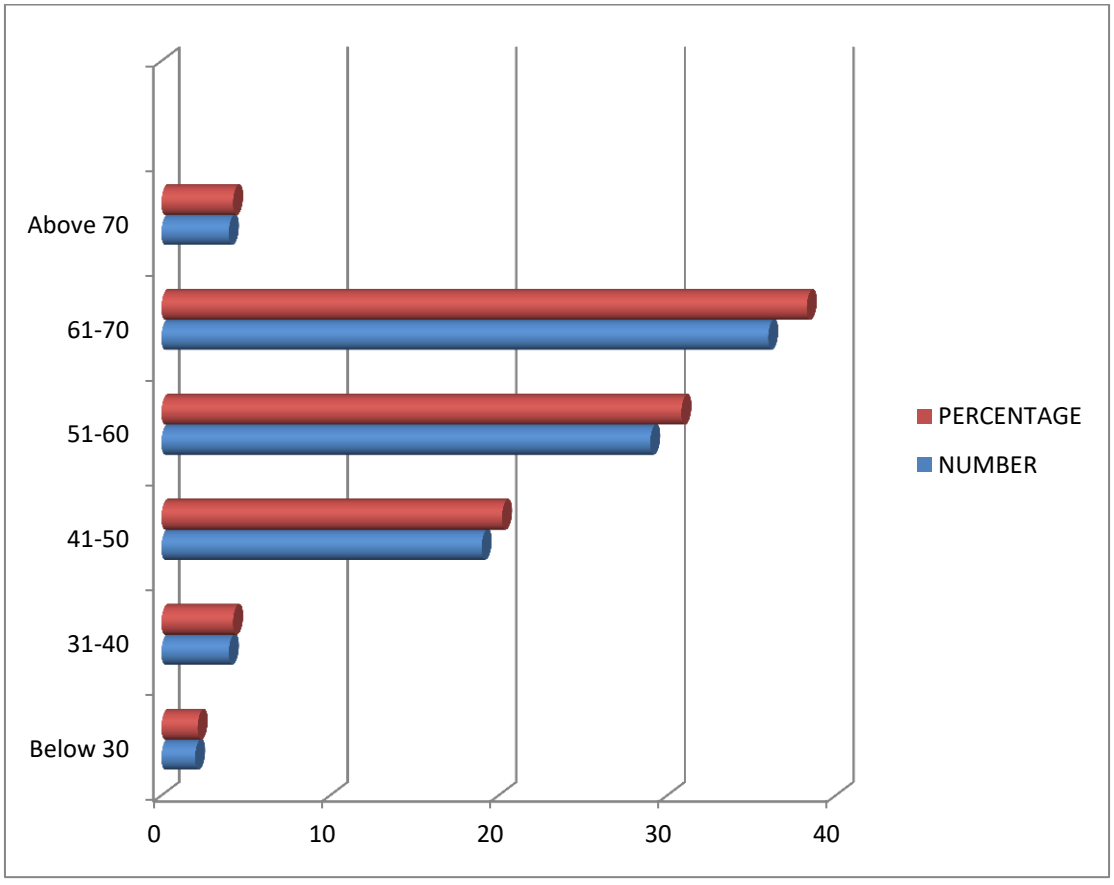
In my study period of two years from July 2015 to August 2017, a prospective study on lung carcinomas conducted in the Department of Pathology Madurai Medical college, Madurai. Among the total 122 lung biopsy specimens received, 14 samples were inadequate for opinion, in the 108 samples processed 94 cases are malignant.

In the present study, the age range is 20-80 years, and the median age is 58 years. Peak incidence for lung carcinomas is 61-70 years. Followed by 51-60 years age group (31%) and least number of tumors in less than 30 years of age group (2.15%).The youngest age of presentation is 20 years. 69 cases are observed in 50 to 70 years, which is 70% of total cases. (TABLE:1, CHART:1).

TABLE:1 AGE WISE DISTRIBUTION OF LUNG CARCINOMAS

AGE	NUMBER	PERCENTAGE
Below 30	2	2.15
31-40	4	4.25
41-50	19	20.21
51-60	29	30.85
61-70	36	38.29
Above 70	4	4.25
TOTAL	94	100%

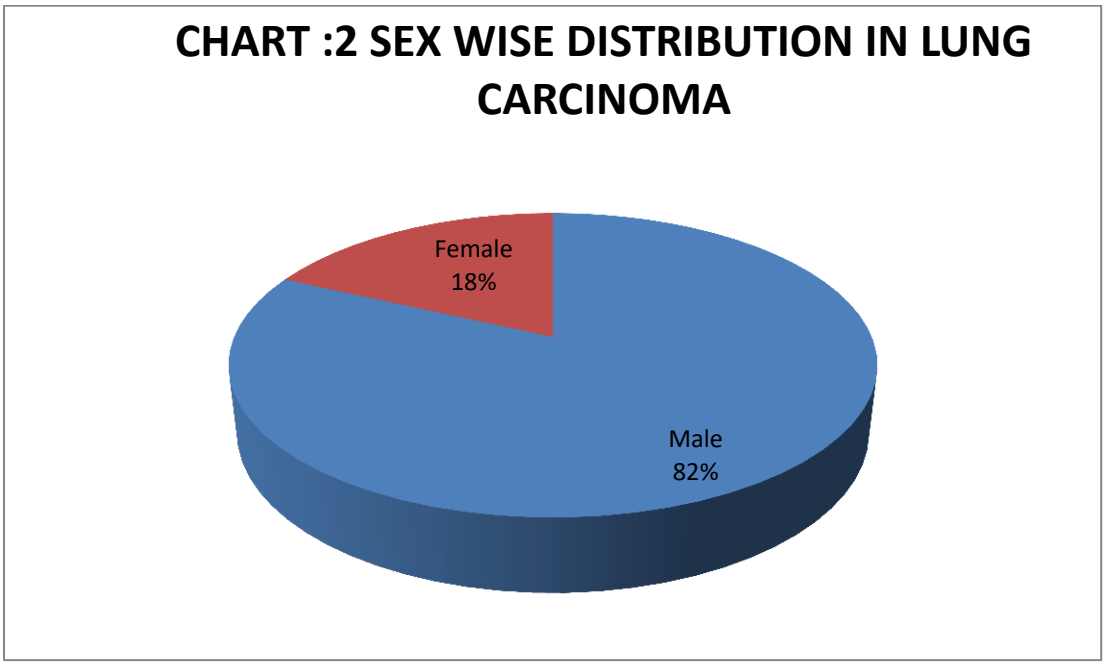
CHART: 1AGE WISE DISTRIBUTION OF LUNG CARCINOMAS



In the present study lung cancer is more common in males with a percentage of 81.7%.With the Male to Female ratio 4.6:1 (TABLE:2, CHART:2).

TABLE:2 SEX WISE DISTRIBUTION OF LUNG CARCINOMAS

GENDER	NUMBER	PERCENTAGE
Male	77	81.7
Female	17	18.3
Total	94	100%

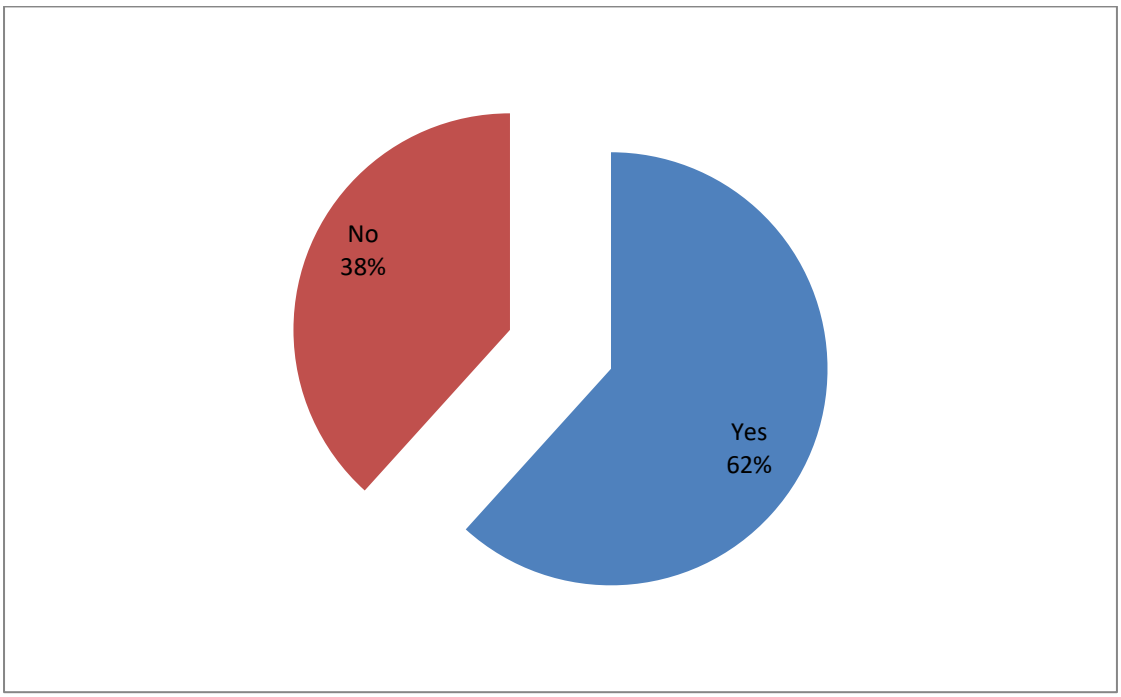


In the present study lung cancer is more common in smokers with a percentage of 61.7%. The Smokers to nonsmokers ratio 2.6:1.The incidence of lung carcinomas is higher in smokers.(TABLE:3,CHART:3)

TABLE:3 DISTRIBUTION OF LUNG CARCINOMAS AMONG SMOKERS & NON SMOKERS

SMOKING HISTORY	NUMBER	PERCENTAGE
Yes	58	61.7
No	36	38.3
Total	94	100%

CHART : 3 DISTRIBUTION OF LUNG CARCINOMAS AMONG SMOKERS & NON SMOKERS

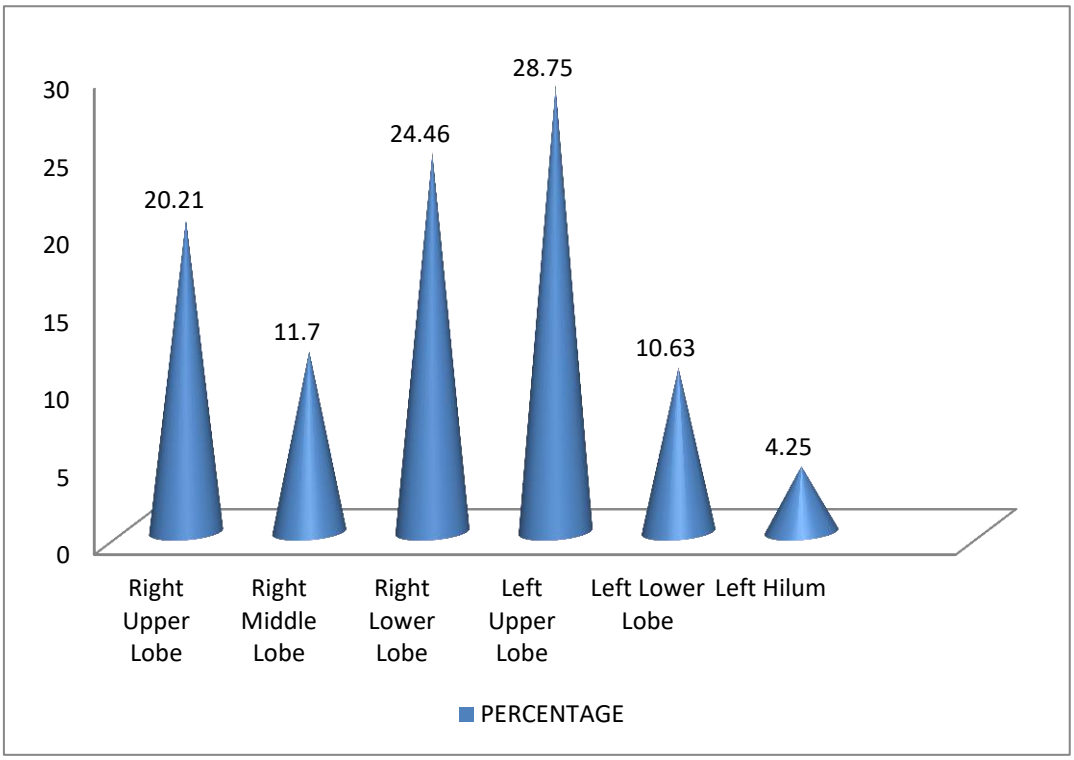


The most common location of tumor is the left upper lobe with incidence of 28.75%. Left lung is more commonly involved than the right lung. (TABLE:4, CHART:4)

TABLE: 4 SITE WISE DISTRIBUTION OF LUNG CARCINOMAS

SITE	FREQUENCY	PERCENTAGE
Right Upper Lobe	19	20.21
Right Middle Lobe	11	11.70
Right Lower Lobe	23	24.46
Left Upper Lobe	27	28.75
Left Lower Lobe	10	10.63
Left Hilum	4	4.25
TOTAL	94	100%

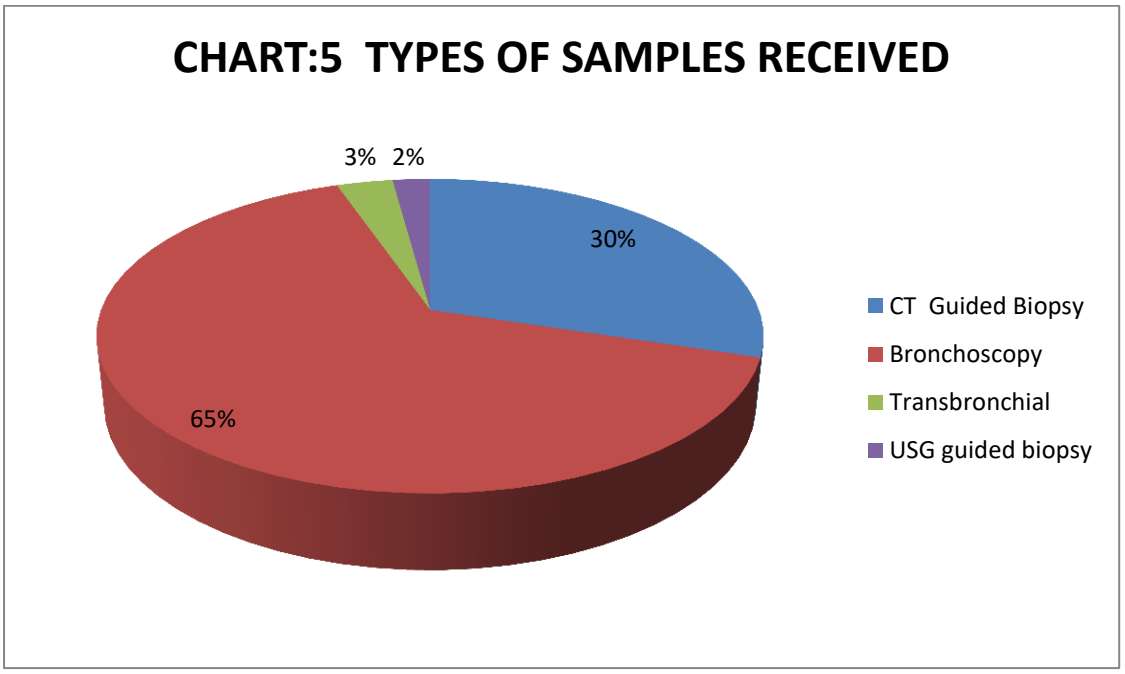
CHART:5 SITE WISE DISTRIBUTION OF LUNG CARCINOMAS



Majority of samples obtained for lung biopsies are by Fiber optic bronchoscopic (FOB) guided biopsy with the highest percentage of (64.89%), less frequent by trans thoracic biopsy(fluoroscopy) and USG guided biopsy (5.32%). (TABLE:5, CHART:5)

TABLE:5 TYPES OF SAMPLES RECEIVED

PROCEDURE	FREQUENCY	PERCENTAGE
CT Guided Biopsy	28	29.78
Bronchoscopy	61	64.89
Transthoracic	3	3.19
USG guided biopsy	2	2.14
TOTAL	94	100

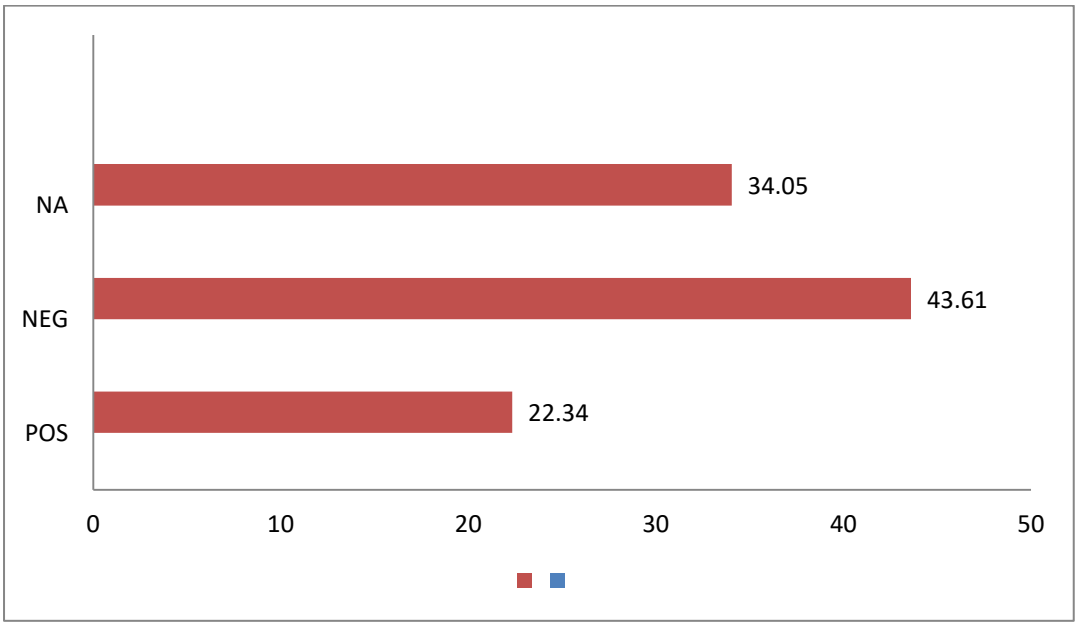


Cytology findings, smears were positive in 21 cases,of about 22.34% and negative in 43.61% mostly due to inadequacy of specimen. (TABLE:6 ,CHART:6)

TABLE:6 CYTOLOGICAL DIAGNOSIS IN LUNG CARCINOMA

CYTOLOGY	FREQUENCY	PERCENTAGE
POS	21	22.34
NEG	41	43.61
NA	32	34.05
TOTAL	94	100

CHART:6 CYTOLOGICAL DIAGNOSIS IN LUNG CARCINOMA



Histologically, the distribution of squamous cell carcinoma and adenocarcinoma, is 59.57% and 24.46% respectively. Squamous cell carcinoma is more common than Adenocarcinoma, NSCLC-NOS accounts for 12.76% of cases and small cell carcinoma 3.21%. (TABLE:8 ,CHART:8)

TABLE:8 HISTOLOGICAL TYPES OF LUNG CARCINOMAS

HPE DIAGNOSIS	NUMBER	PERCENTAGE
Squamous cell Carcinoma	56	59.57
Adenocarcinoma	23	24.46
NSCLC- NOS	12	12.76
Small cell Carcinoma	3	3.21
TOTAL	94	100

CHART:8 HISTOLOGICAL TYPES OF LUNG CARCINOMAS

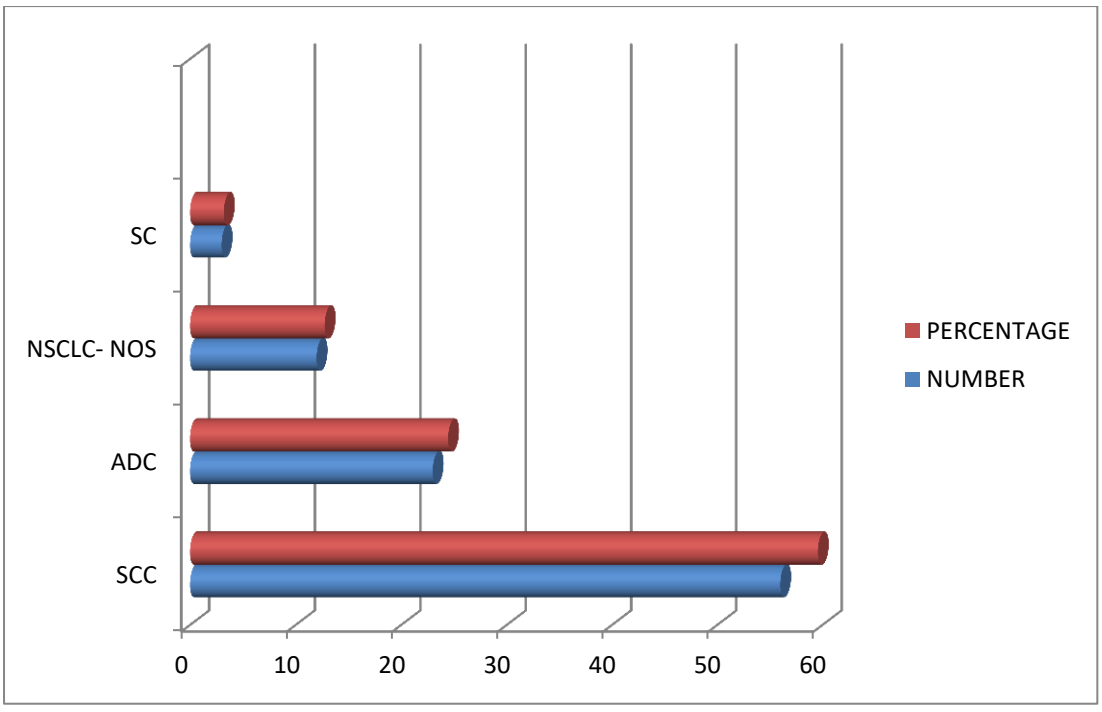


TABLE:9 AGE WISE DISTRIBUTION OF LUNG CARCINOMAS - HISTOLOGICAL TYPES

AGE	SCC	ADC	NSCLC-NOS	SC
Below 30	1	-	-	1
31-40	2	1	-	1
41-50	11	5	5	1
51-60	17	8	2	-
61-70	21	8	5	
Above 70	4	1	-	-
TOTAL	56	23	12	3
PERCENTAGE	59.57%	24.46%	12.76%	3.21%

Sex wise distribution of non small cell lung carcinomas 81.7% of the cases are men and 18.3% of the cases are women with increased incidence of squamous cell carcinoma in men and adenocarcinoma seen more commonly in women.(TABLE:10)

TABLE:10 SEX WISE DISTRIBUTION OF LUNG CARCINOMAS - HISTOLOGICAL TYPES

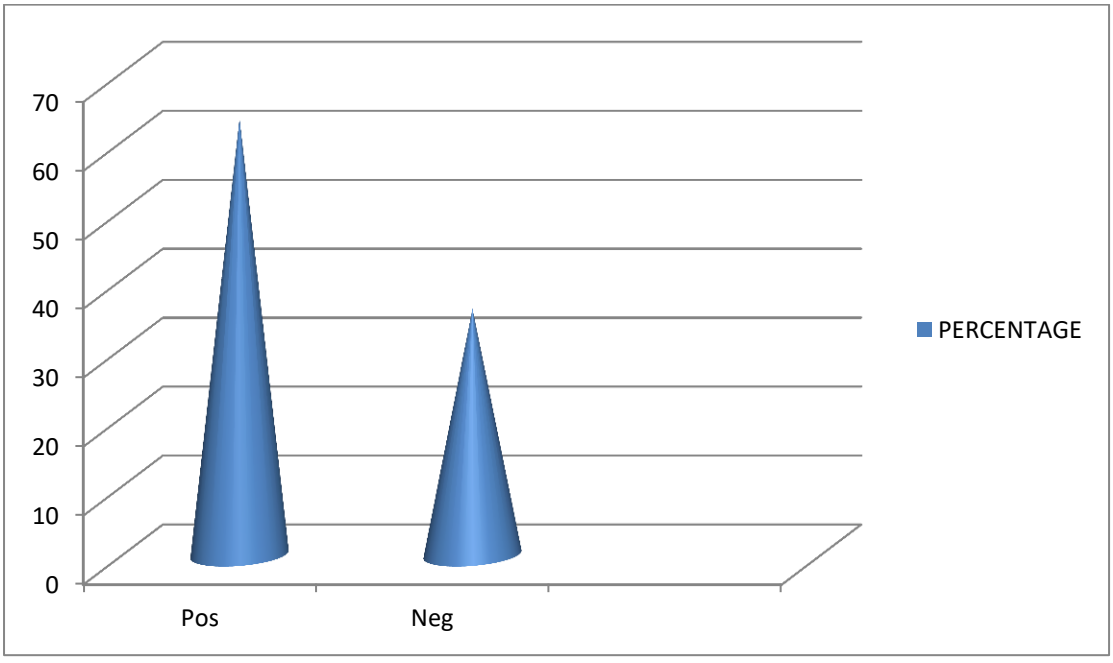
SEX	SCC	SCC%	ADC	ADC%	NSCLC-NOS	NSCLC-NOS%	SC	SC%
MALE	46	48.93%	17	18.08%	11	11.70%	3	3.19%
FEMALE	10	10.63%	6	6.38%	1	1.06%	-	0

In the present study IHC for EGFR is done for randomly selected 10 cases each of SCC, Adenocarcinoma, NSCLC-NOS respectively. EGFR is positive in 19 cases constituting 63.33%.Negative in 11 cases with a percentage of 36.67.

TABLE 11: DISTRIBUTION OF EGFR POSITIVITY

EGFR	NUMBER	PERCENTAGE
Pos	19	63.33
Neg	11	35.67
TOTAL	30	100

CHART 9: DISTRIBUTION OF EGFR POSITIVITY

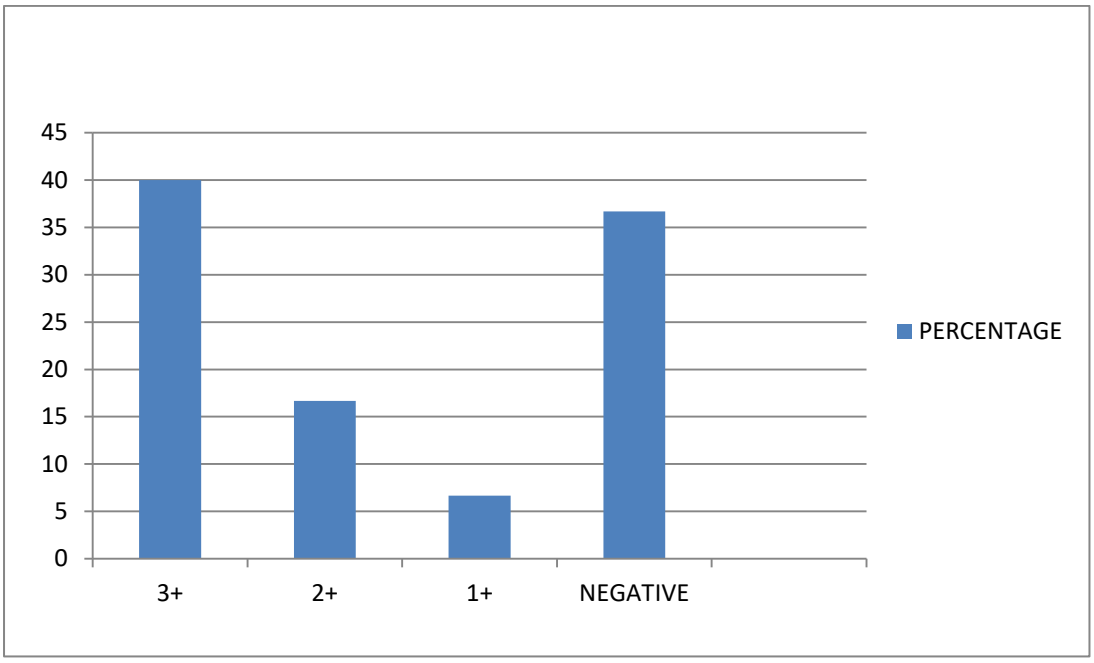


EGFR is graded according to the percentage of positive tumor cells and the intensity of staining .Accordingly it is graded as Negative, 1+, 2+ and 3+. Twelve (n=12) cases showed 3+ grade with a percentage of 40%.5 cases showed 2+ grade with a percentage of 16.66%.Two cases (n=2) showed grade 1+ with percentage of 6.66%.

TABLE 12 : DISTRIBUTION OF EGFR GRADING

EGFR	FREQUENCY	PERCENTAGE
3+	12	40
2+	5	16.66
1+	2	6.66
NEGATIVE	11	36.68
TOTAL	30	100

CHART 10: DISTRIBUTION OF EGFR GRADING



SEX WISE DISTRIBUTION OF EGFRIN THE PRESENT STUDY:

In the present study EGFR expression is increased in females with 84.62%, males with a percentage of 47.05%. 15.38% of females were negative for EGFR expression, 52.95 % in males. (TABLE:13)

TABLE 13:SEX WISE DISTRIBUTION OF EGFR

EGFR	MALES	MALES PERCENTAGE	FEMALES	FEMALES PERCENTAGE	TOTAL
POSITIVE	8	47.05	11	84.62	19
NEGATIVE	9	52.95	2	15.38	11
TOTAL		100		100	30

The EGFR expression is compared with smokers and non smokers. It is estimated that 8 smokers were positive and 9 were found to be negative for EGFR expression. 11 nonsmokers were positive for EGFR, 2 cases found to be negative.

This is statically significant in the present study p value of 0.034405 ($p<0.05$)

TABLE 14: DISTRIBUTION OF EGFR IN SMOKERS AND NONSMOKERS

EGFR	YES	% OF SMOKERS	NO	% OF NON SMOKERS	TOTAL
POSITIVE	8	47.05	11	84.62	19
NEGATIVE	9	52.95	2	15.38	11
TOTAL	17	100	13	100	30

The distribution of EGFR in various histological types of non small cell lung carcinomas, the results are in the present study, EGFR expression is more commonly seen in adenocarcinoma with a percentage of 47.36%. 31.57% of squamous cell carcinomas are positive for EGFR expression and 21.07% of NSCLC-NOS are positive for EGFR expression.(TABLE :15)

TABLE 15: DISTRIBUTION OF EGFR IN NON SMALL CELL LUNG CARCINOMAS

HPE DIAGNOSIS	EGFR POSITIVE	EGFR POSITIVE%	EGFR NEGATIVE	EGFR NEGATIVE%	TOTAL CASES
ADC	9	47.36	1	9.09	10
SCC	6	31.57	4	36.36	10
NSCLC-NOS	4	21.07	6	54.55	10
TOTAL	19	100	11	100	30

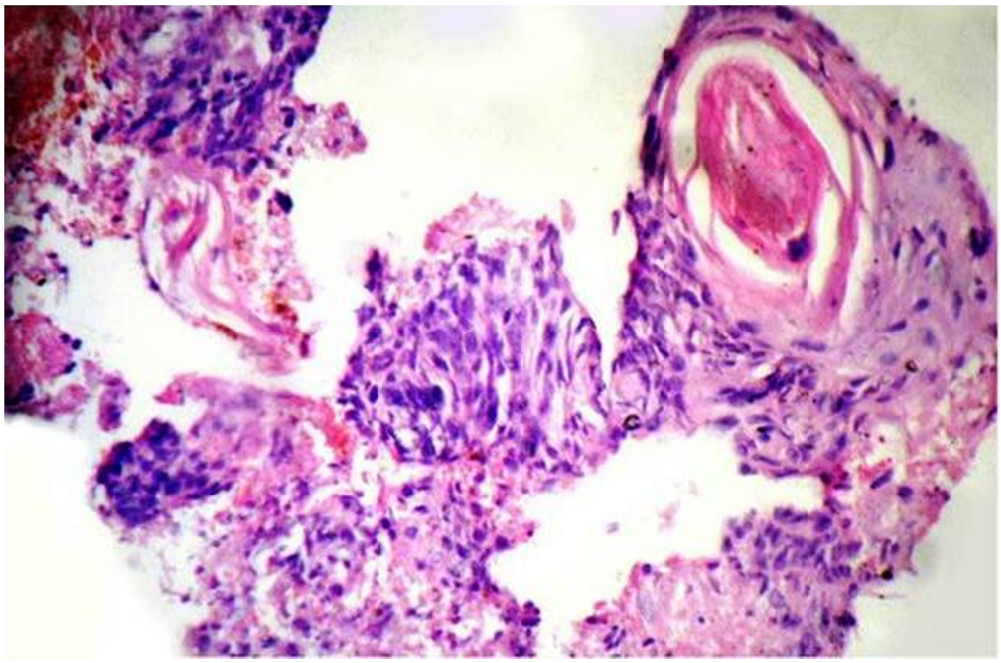


FIGURE 1 : WELL DIFFERENTIATED SQUAMOUS CELL CARCINOMA (40X HPE) HPE NO: S 1376/16

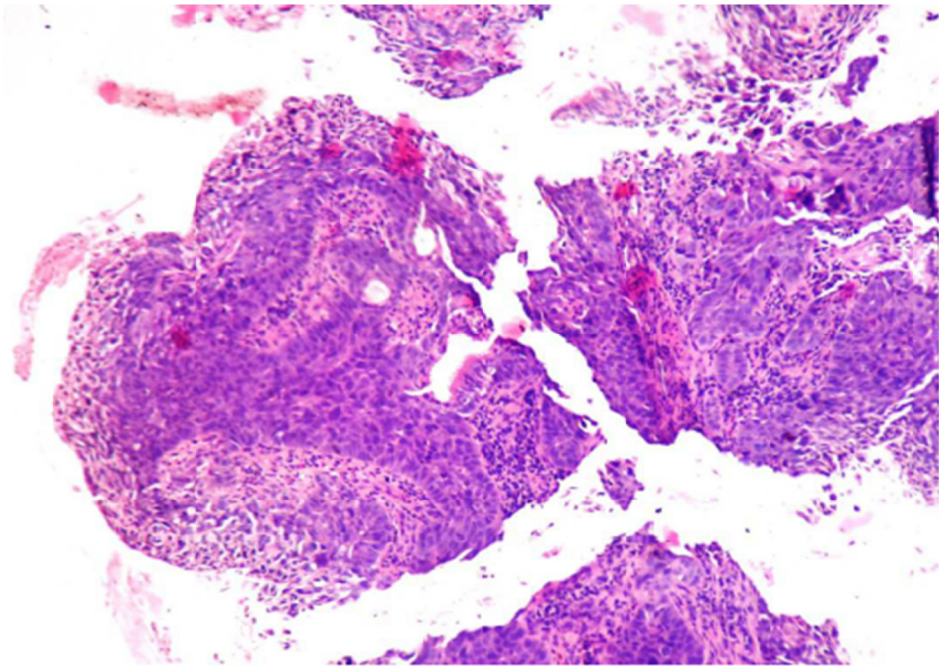


FIGURE 2: MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA 100X HPE NO: S1151 /16

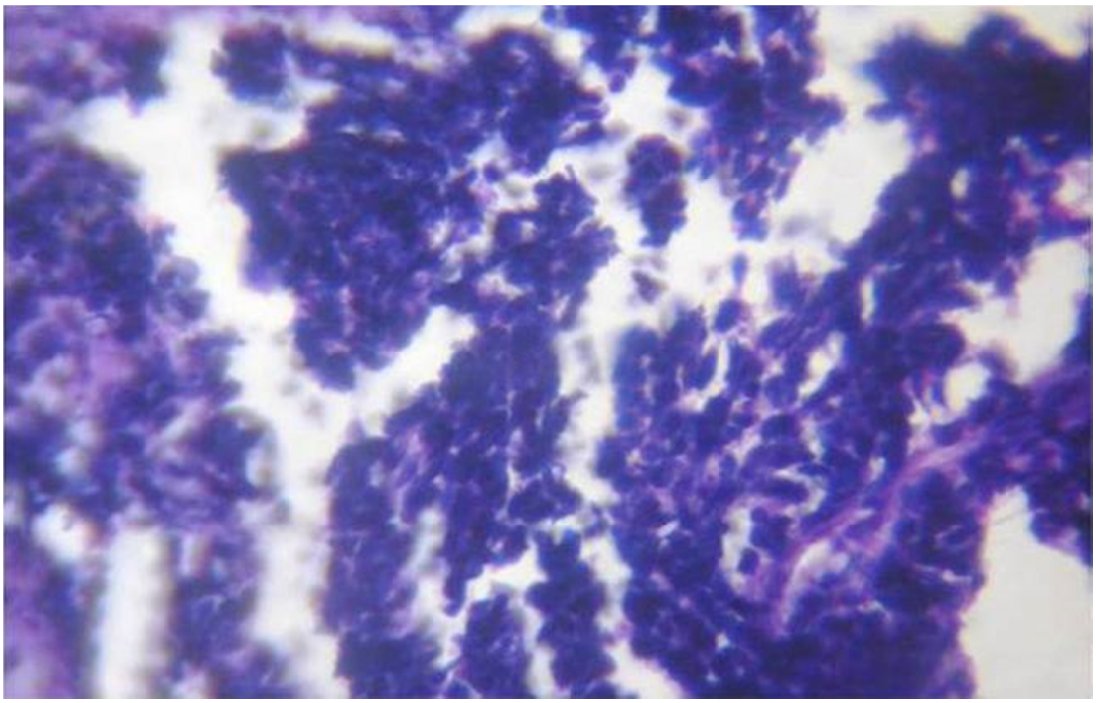


FIGURE 3: SMALL CELL CARCINOMA 100X HPENO: S 1029/ 16

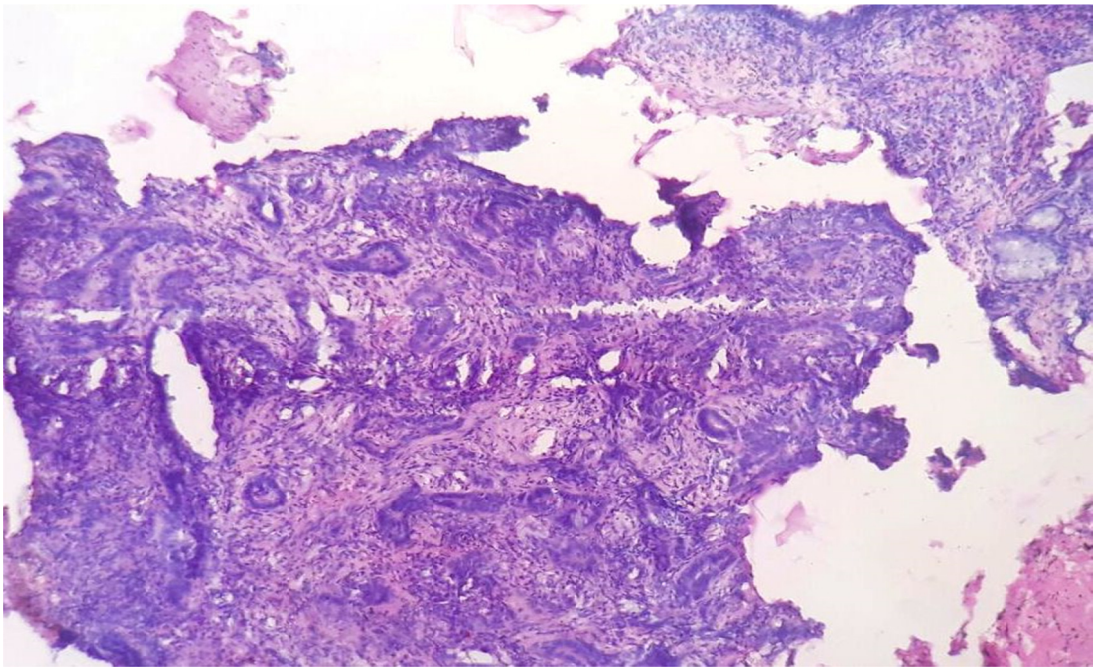


FIGURE 4: WELL DIFFERENTIATED ADENOCARCINOMA 40X HPE NO: S 1972 /16

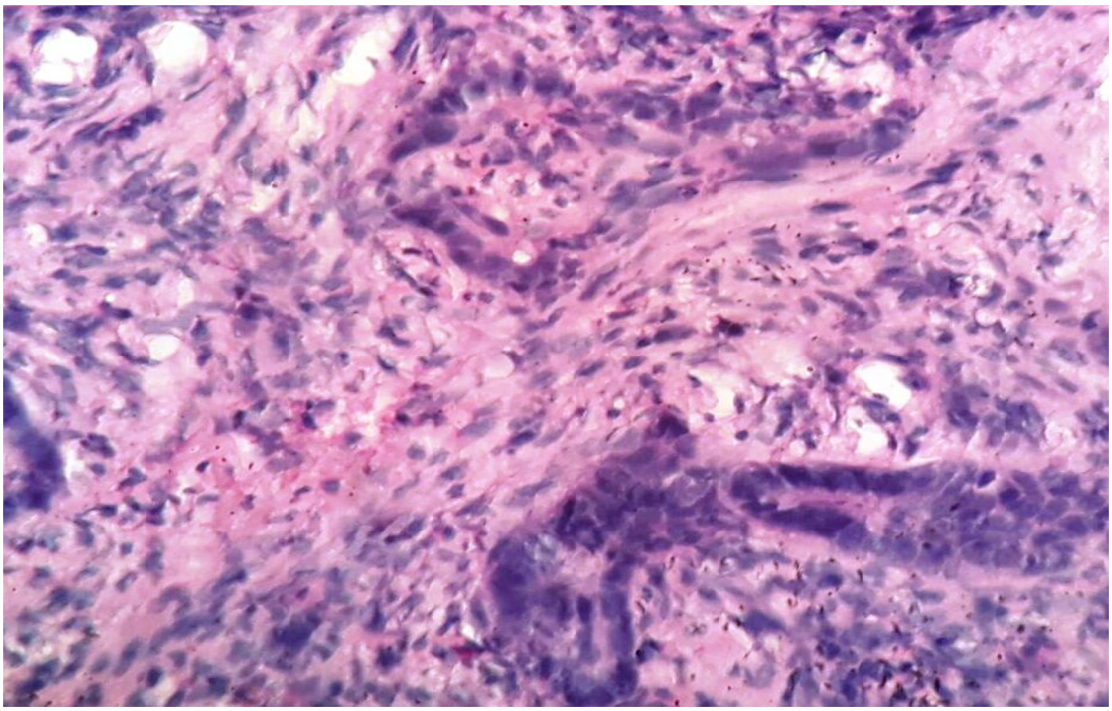


FIGURE 5: WELL DIFFERENTIATED ADENOCARCINOMA 100X HPE NO: S1972/16

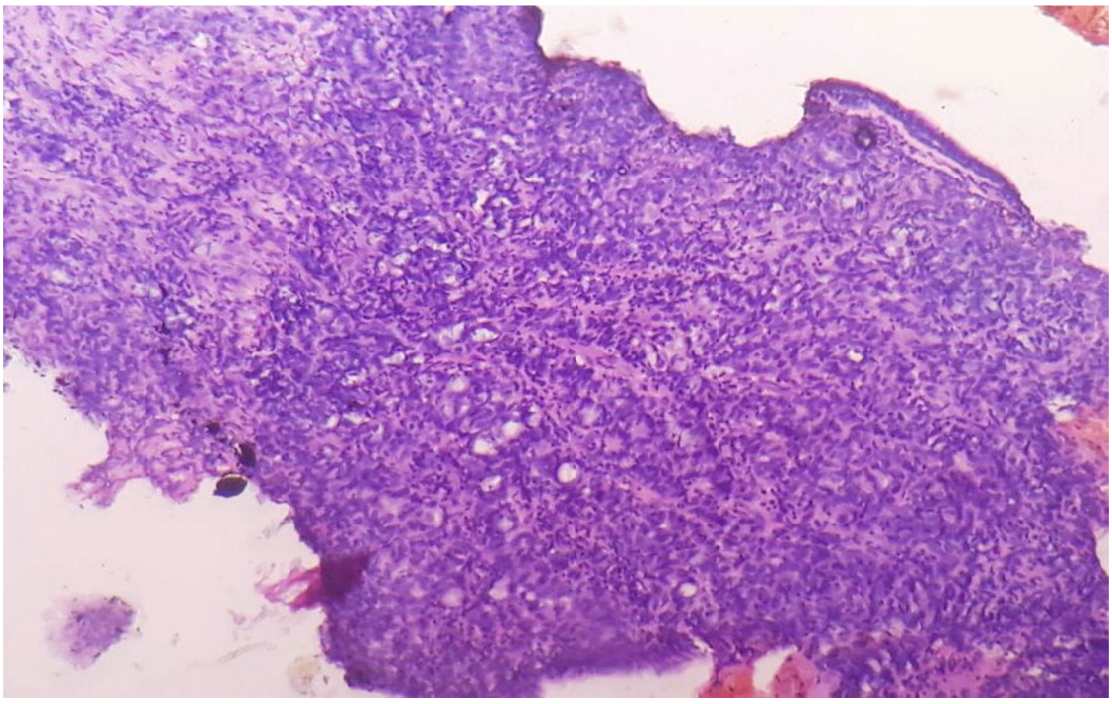


FIGURE 6: MODERATELY DIFFERENTIATED ADENOCARCINOMA 40X

HPENO: S 1301/16

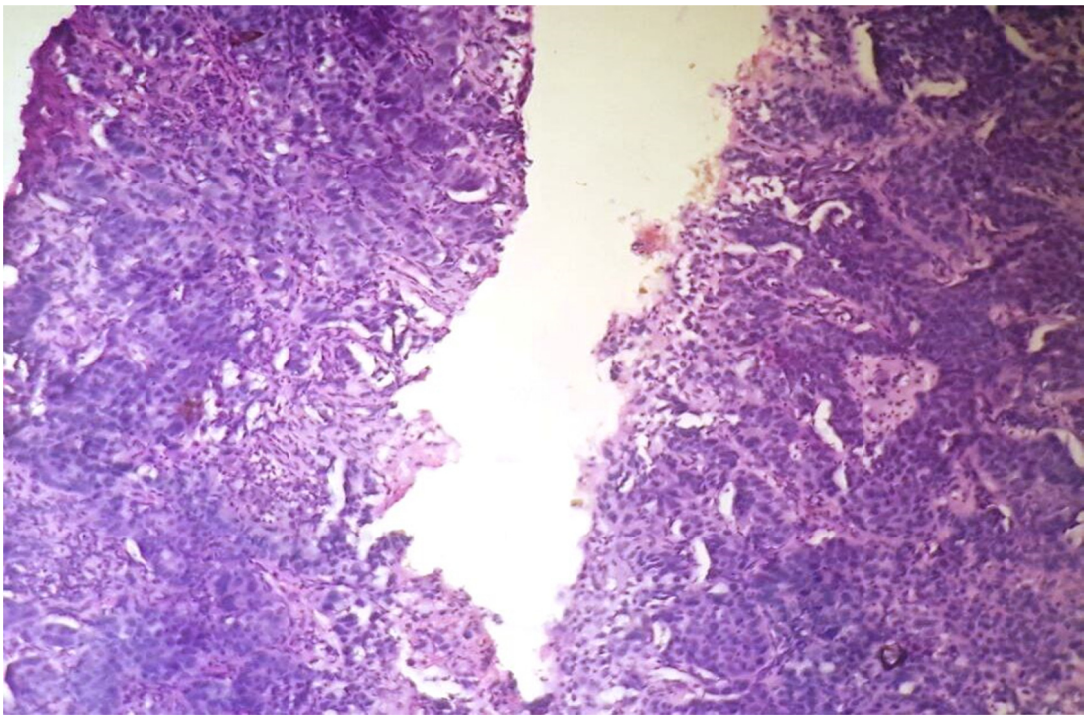


FIGURE 7: POORLY DIFFERENTIATED ADENOCARCINOMA 10X – HPE NO: S 1642/16

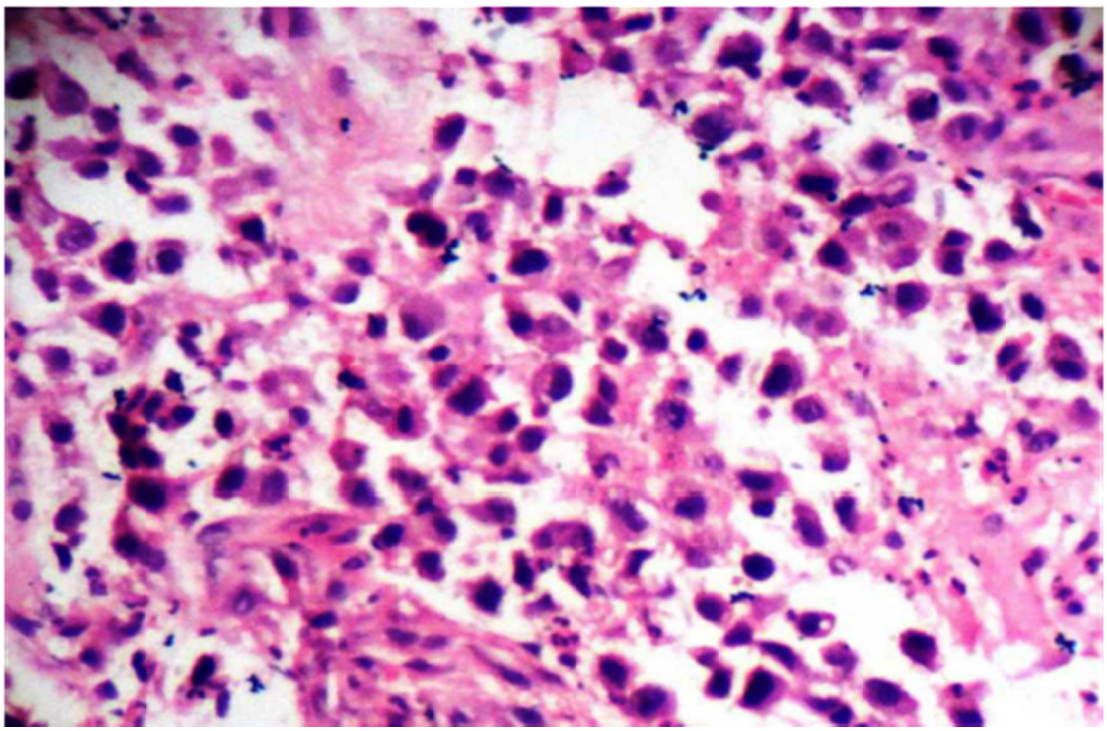


FIGURE 8: NON SMALL CELL LUNG CANCER -NOS – 10X HPE

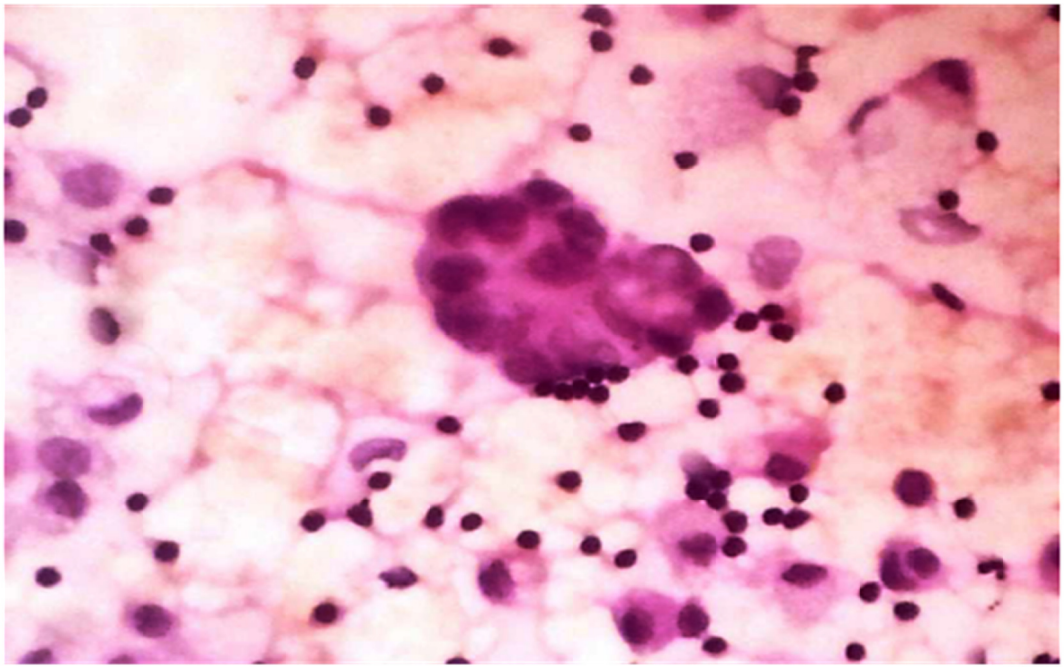


FIGURE 9: BRONCHIAL WASH CYTOLOGY -SMEARS POSITIVE FOR MALIGNANT CELLS (ADENOCARCINOMA) - 40X HPENO: S1329/17

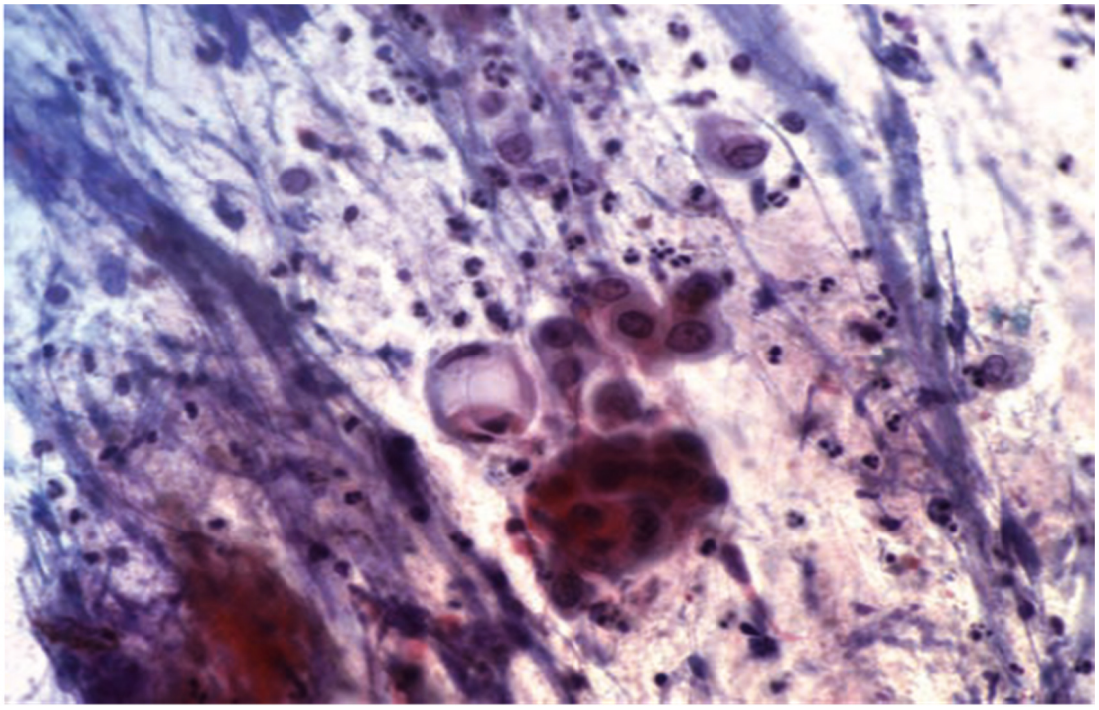


FIGURE 9: BRONCHIAL WASH CYTOLOGY -SMEARS POSITIVE FOR MALIGNANT CELLS (SQUAMOUS CELLCARCINOMA) -40X HPENO: S1089/ 17

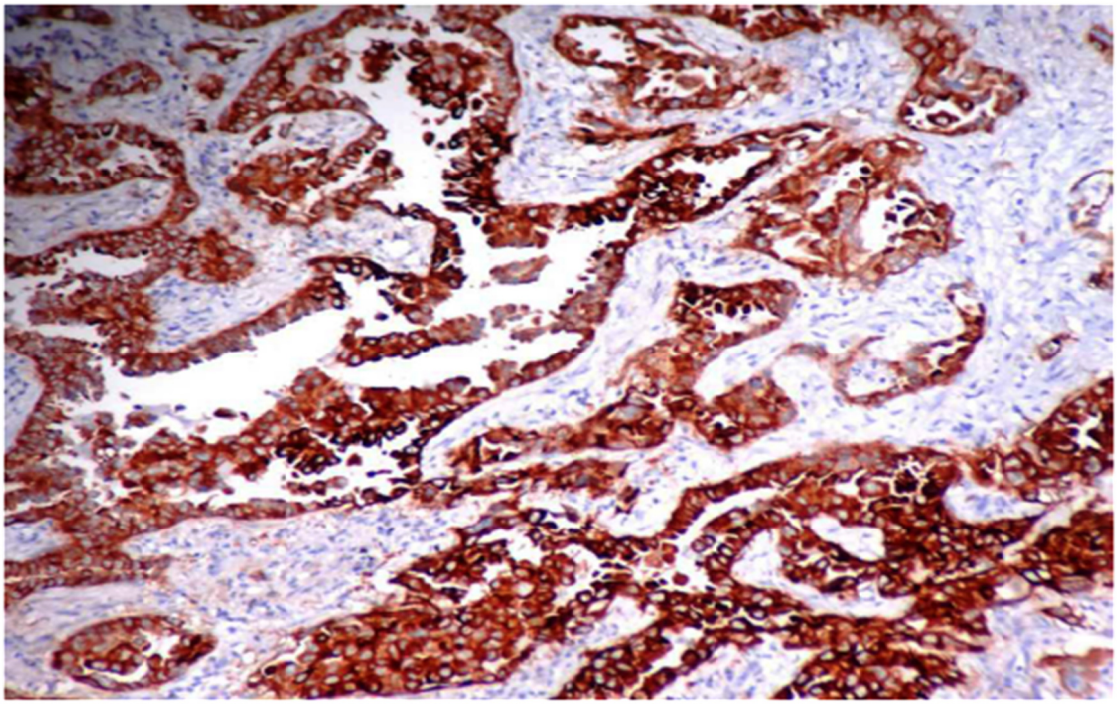


FIGURE 11: EGFR POSITIVITY IN ADENOCARCINOMA – GRADE 3 10X HPE NO: S 1725/16

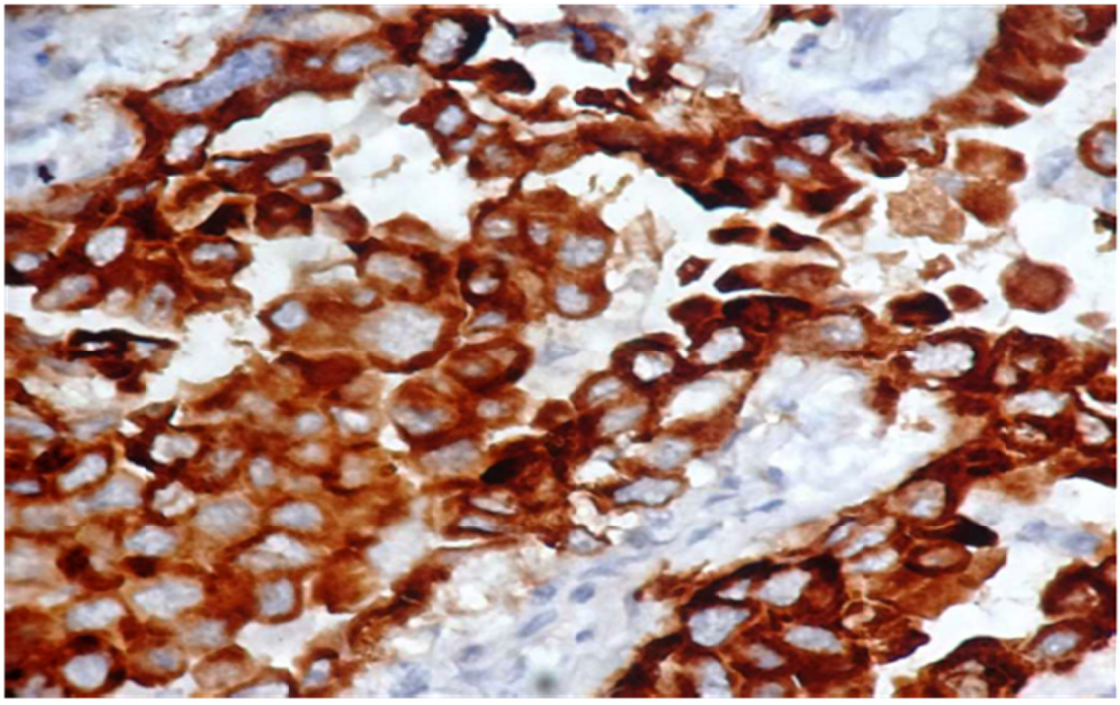


FIGURE 12: EGFR POSITIVITY IN ADENOCARCINOMA – MEMBRANE STAINING 40X HPENO: S1725/16

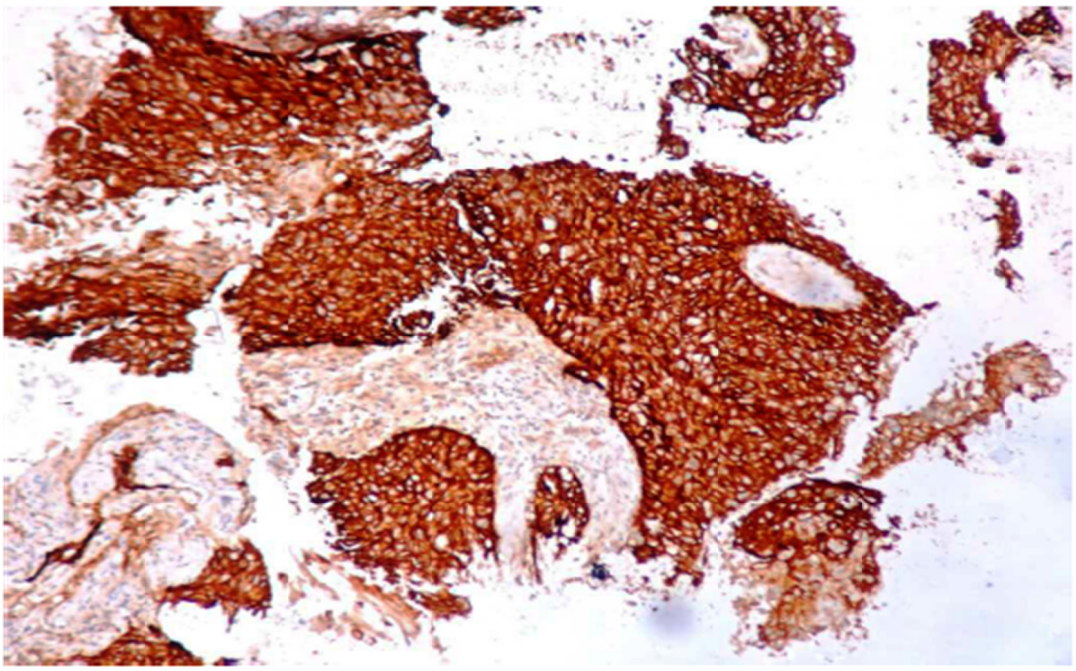


FIGURE 13: EGFR POSITIVITY IN NSCLC -NOS GRADE-3 10X

HPENO: S 1147/17

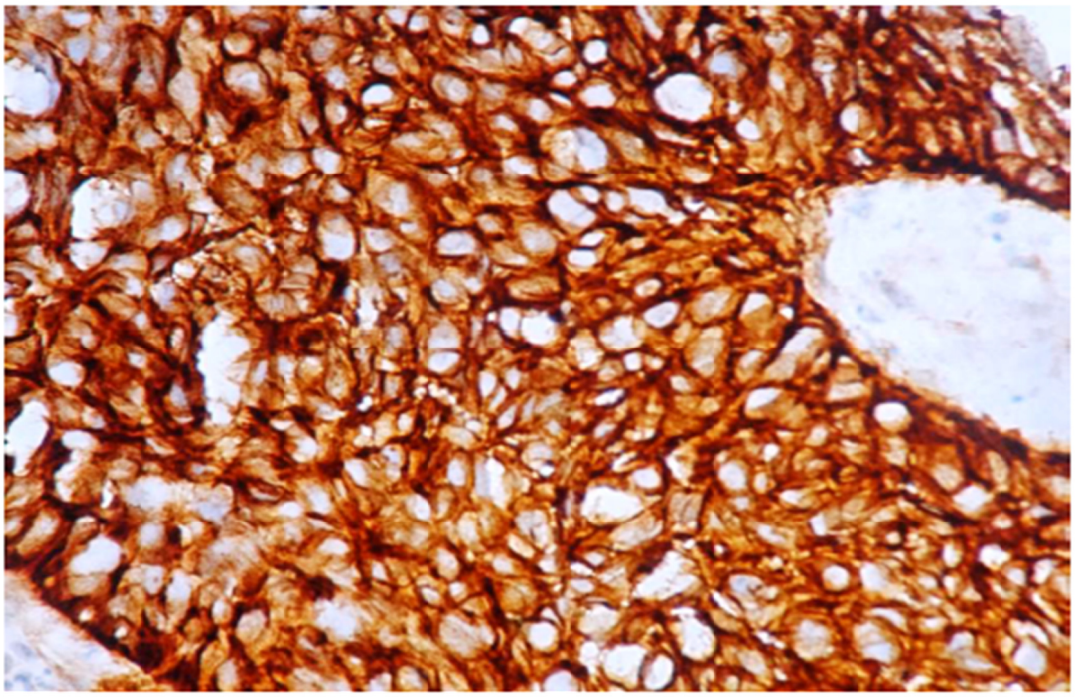


FIGURE 14: MEMBRANE POSITIVITY- 40X HPENO: S 1147/17

DISCUSSION

DISCUSSION

Lung carcinoma constitutes 11% of all cases of carcinomas and it constitutes around 13% of all cancer related mortality. It is more predominant in males 5^{th} to 7^{th} decade of life. Also the prognosis of lung cancer is very poor.

Among the histological types of lung carcinoma, more than 85% are non small cell lung cancer. Non small cell lung cancer has to be sub classified accurately into squamous cell carcinoma, adenocarcinoma, non small cell lung cancer-NOS. Squamous cell carcinoma is the most common type of lung carcinoma followed by adenocarcinoma.

Until the recent past, the histological sub classification has no clinical or therapeutic significance. Most of the lung carcinomas are in advanced stage at presentation. The surgical cure rate is very low, probably less than 10% of cases. With the advent of targeted therapies, it is essential to subtype non small cell lung cancer as it has direct effect on treatment and prognosis.

It is necessary to identify the EGFR expression in adenocarcinoma and other type of lung cancers as EGFR tyrosine kinase inhibitors forms the primary treatment.

Madurai medical college is a tertiary care centre, this is a descriptive study of lung cancers conducted for two years between July 2015 to August 2017. A total of 122 lung cancer specimens were found in this study period. More than 95% of lung cancers in the present study is Non small cell lung cancer. Small cell lung carcinoma accounts for 3.19% of cases. This is in

accordance with the study done by Ruquia Afrose et al[92]which states that around 80-85% of lung cancer cases are non small cell lung carcinoma and thus it is the most common type.

Most of the lung cancers present in the age group between 50-70 years of age with 70% incidence, less than 2.12% of cases are seen in the age group less than 30 years. This is in correlation with the study done by Navin Pandhi et al[91]and others. The median age group in our study is 58 years.

Median age of lung carcinoma in various studies compared with the current study,

STUDY	MEDIAN AGE
Navin Pandhi et al	59
Ruquia Afrose et al	56
Ayandip Nandi et al	54
Jing C et al	62
Present study	58

In our study lung cancer is more common in males with a percentage of 81.7%. It is observed that the incidence of lung cancer in squamous cell carcinoma is more common among males. But females are more commonly affected by adenocarcinoma than by squamous cell carcinoma.

This is accordance with the study conducted by Ruquia Afrose et al[92]. A study by Kiyohara et al[102]has estimated that the ability of DNA repair is relatively low in females when compared to males and they are said to have increased susceptibility for lung cancer.

Percentage of gender distribution in other studies compared with the current study,

STUDY	MALE FEMALE RATIO
Navin Pandhi et al	2.7 :1
Ruquia Afrose et al	4.5 :1
Ayandip Nandi et al	4.2:1
Jing C et al	3.2 :1
Present study	4.6 :1

In our study 62% of cases are smokers and 38% of the cases are nonsmokers. Squamous cell carcinoma is strongly associated with smoking with 48.93%.And most of the adenocarcinoma patients are non smokers with 35%. This is in accordance with the study conducted by Thompson TG et al[103]who estimated that smoking is the most common cause for both males and females and it is more strongly associated with squamous cell carcinoma.

Comparison of smoking incidence in various studies with the current study

Studies Cases (n) Smokers (%) Nonsmokers (%)

STUDY	CASES(n)	Smokers	Non Smokers
Navin Pandhi et al	150	90(60%)	60(40%)
Ruquia Afrose et al	342	260(76%)	82(24%)
Ayandip Nandi et al	80	62(78%)	18(22%)
Jing C et al	120	98(82%)	22(18%)
Present study	94	58(62%)	36(38%)

In our study, Squamous cell carcinoma is the most common histological subtype of lung carcinoma with 59.57% followed by adenocarcinoma which is 24.46%.Although historically SCC is the most common subtype, the recent study by Tieulent-et al[(104)]in 2014 has stated that adenocarcinoma incidence is on the increasing side and it has surpassed those of SCC.

Comparison of histological subtypes with various studies:

HISTOLOGICAL SUBTYPE	Navin Pandhi et al	Ruquia Afrose et al	Present study
Squamous cell carcinoma	46%	54%	59.57%
Adenocarcinoma	31%	27%	24.46%
NSCLC-NOS	13%	14.6%	12.76%
Small cell carcinoma	10%	4.4%	3.21

Squamous cell carcinoma:

In our study, squamous cell carcinoma is the most common type of non small cell lung cancer. It is diagnosed histologically based on the keratin formation and the appearance of intercellular bridges. Among the 94 malignant cases in our study, 56 cases come under this category with a percentage of 59.57% and showed a male predominance with 46 cases with a percentage of 60%.

Out of the total 56 cases of squamous cell carcinoma, 46 cases are smokers with a percentage of 82.14%, 17.86% of cases are non smokers.

Adenocarcinoma :

In our study, adenocarcinoma is the second most common subtype. The presence of glandular features and mucin production are the characteristic features of this subtype.

Out of the total 94 malignant cases, 24.46% of cases come under this category (n=23). Out of the 23 cases 17 cases are males and 6 cases are females with 73.91% and 26.09% respectively.

Left upper lobe is the most common site of involvement in our study with 28.72% (n=27). It is followed by right lower lobe with 24.46%. The right lung is more commonly involved than the left lung with 56.38% and 43.26% respectively

Expression of EGFR:

EGFR plays an important role in cell proliferation, invasion and angiogenesis of tumor cells. It is thus considered to be a poor prognostic factor for survival in non small lung cancer.

EGFR positivity among non small cell lung cancers:

In the present study randomly selected 30 cases of non small cell lung cancer,63.33 % of Cases are positive for EGFR expression. The various studies results are as follows,

EGFR positivity

STUDY	EGFR POSITIVITY
Brevet et al	69.7%
Yong Hannahwen et al	48.6%
Gao J et al	36.7%
Yuan yang et al	33.7%
Present study	63.33%

Comparison of EGFR expression in males and females in other studies with the current study:

In the present study, EGFR seems to be increasingly expressed among females with a percentage of 84.62. 47.05% of the males are positive for EGFR expression. This is in accordance with the study done by Brevet et al, states that EGFR expression is significantly high in females.

The results are statistically significant in the present study with the p value of 0.034405 ($p<0.05$)obtained by t test.

EGFR expression in males and females in other studies compared with our Study

STUDY	FEMALES	MALES
Brevet et al	63.7%	49.3%
Yong Hannahwen et al	47.5%	15%
Yuan yang lai et al	57.5%	22.7%
Present study	84.62%	47.05%

Comparison of EGFR expression among smokers and non smokers:

It is estimated in our study that 84.62% of the EGFR positive cases are Nonsmokers. This is in accordance with the study conducted by Yaxiong Zhang etal. This is statically significant in the present study p value of 0.035604 ($p<0.05$) obtained by t test.

STUDY	NON SMOKERS	SMOKERS
Brevet et al	42.3%	13.9%
Yong Hannah wen et al	40.9%	12.4%
Yuan yang et al	39.5%	30.3%
Present study	84.62%	47.05%

Age wise distribution of EGFR:

In the present study, 38.29% of the EGFR positive patients are in the age group between 61 and 70 years. 65.31% of the cases are more than 60 years of age and 34.69% of the patients are less than 60 years.

The study by Yuan yang et al that 37.3% of the patients are above 60 years of age and 30.7% of the patients are less than 60 years of age. Study by Feng Q et al has suggested that most EGFR positive patients are less than 60 years.

EGFR expression in various histological subtypes:

In the present study, EGFR expression is more commonly seen in adenocarcinoma with a percentage of 47.36%. 31.57% of squamous cell carcinomas are positive for EGFR expression and 21.07% of NSCLC-NOS are positive for EGFR expression. The comparison in NSCLC and EGFR POSITIVITY with various studies are as follows,

STUDY	ADC	SCC	NSCLC-NOS
Brevet et al	61.1%	29.7%	40%
Vamitha et al	43.90%	36.59%	19.51%
Present study	47.36%	31.57%	21.07%

The comparison of EGFR distribution with various histological subtypes is statistically significant in our study with p value of0.037021 ($p<0.05$).

LIMITATIONS OF THE STUDY

- The patients are selected from the tertiary care centre and the study population might not represent the general population.
- DNA sequencing gives more accurate report than the immunohistochemistry. Since it is expensive, it cannot be applied to all the patients.
- Follow up of the patients is not complete thus the prognostic influence cannot be ascertained.

SUMMARY

SUMMARY

This study on lung carcinomas is a prospective study conducted in the Department of Pathology, MADURAI MEDICAL COLLEGE, Madurai during the period of July 2015 to August 2017.

- Among the total 122 lung biopsy specimens received, in 14 cases the sample was inadequate for opinion, in the 108 samples processed 94 cases were malignant.
- Among the types of small biopsies received are, FOB guided biopsy contributed to a majority of (64.89%), trans thoracic biopsy and USG guided biopsy(5.32%).
- The mean age of presentation of lung cancer was 58 years. The youngest age at presentation was 20 years.
- Among the 94 malignant cases, 81.7% of the cases were men and 18.3% of the cases were women.
- 62% of the cases was smokers and 38% of the cases nonsmokers, with strong smoking association in squamous cell carcinoma.
- The most common location of tumor was the left upper lobe. Left lung was more commonly involved than the right lung.
- Among the 94 malignant cases, 91 cases were non small cell lung cancer which vastly outnumber small cell lung carcinoma cases.
- According to WHO classification, the distribution of squamous cell carcinoma and adenocarcinoma, was 59.57% and 24.46% respectively.

Squamous cell carcinoma was more common than Adenocarcinoma, NSCLC-NOS accounts for 12.76% of cases.

- With 81.7% of the cases were men and 18.3% of the cases were women with increased incidence of squamous cell carcinoma in men and adenocarcinoma seen more commonly in women.
- 62% of the cases were smokers with strong smoking association in squamous cell carcinoma with 48.93%
- Thirty cases of non small cell lung cancer are randomly selected from each category for EGFR expression by IHC. 63.33% of the cases are positive for EGFR expression.
- 84.62% of the females are positive for EGFR expression and 47.05%of the males showed positivity with p value- 0.034405($p<0.05$)
- 81.62% of the EGFR positive cases are non smokers and 45.05% are smokers. p value- 0.035604($p<0.05$)
- 38.29% of the EGFR positive patients are in the age group between61 and 70 years.
- Among the EGFR positive histological subtype, adenocarcinoma is the most common type with 47.36% followed by squamous cell carcinoma with 31.57 % & NSCLC-NOS with 21.07 %. p value- 0.037021($p<0.05$)

CONCLUSION

CONCLUSION

Vast majority of the lung carcinoma cases present at advanced stage, the introduction of targeted therapies particularly EGFR expression responding for tyrosine kinase inhibitors has revolutionised the treatment of lung cancer patients.

The identification of EGFR expression gives a better opportunity for the treatment with tyrosine kinase inhibitors against non small cell lung cancers. It is evident from the comparison of various studies with present study that EGFR expression is more common in females, nonsmokers and adenocarcinoma histological type.

EGFR expression, being a poor prognostic factor, its expression is essential to identify the tyrosine kinase inhibitors sensitivity.

Hence it is very important to find the association between EGFR expression and its clinocopathological parameters in order to select the patients for targeted therapy like erlotinib, gefitinib for advanced lung cancers. More recently afatinib, tyrosine kinase inhibitors (TKI) drug approved for the treatment of patients with lung adenocarcinoma, have been shown to significantly extend progression-free and overall survival in patients those harbor activating EGFR mutations.

In conclusion, it is essential that EGFR expression should be assessed after lung biopsy for non small lung carcinomas especially adenocarcinoma and adenosquamous carcinoma for better management of the patients.

ANNEXURES

ANNEXURE - I

ABBREVIATION

H & E: Hematoxylin & Eosin

ADC: Adenocarcinoma

SCC: Squamous cell carcinoma

NSCLC: Non Small Cell Lung Carcinoma

NSCLC-NOS: Non Small Cell Lung Carcinoma-not otherwise Specified

CIS: Carcinoma in situ

BAC**:** Bronchoalveolar Carcinoma

AIS: Adenocarcinoma Insitu

MIA: Minimally Invasive

WHO: World Health Organisation

IASLC/ATS/ERS: International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society

IHC: Immunohistochemistry

TTF-1: Thyroid Transcription Factor-1

EGFR: Epidermal growth factor receptor

ANNEXURE - II

PROFORMA

Name: Age / Sex:

IP No: Unit & Ward:

HPE No:

H/O Presenting illness:

Significant past history (if any):

Type of biopsy specimen: Fibreoptic Bronchial , CT guided , Trans thoracic, USG Guided

Anatomical site & Laterality: Right upper lobe/middle lobe/lower lobe/ hilum

Left upper lobe/lower lobe/hilum

Details of any relevant imaging studies:

Details of Bronchoscopy findings:

Details of any investigation for metastatic disease:

ANNEXURE III

WHO CLASSIFICATION OF LUNG CARCINOMA

Malignant epithelial tumors

Adenocarcinoma

Lepidic adenocarcinoma

Acinar adenocarcinoma

Papillary adenocarcinoma

Micro papillary adenocarcinoma

Solid adenocarcinoma

Adenocarcinoma, mixed subtype

Nonmucinous

Invasive Mucinous

Mixed nonmucinous and mucinous or indeterminate

Fetal adenocarcinoma

Enteric adenocarcinoma

Mucinous (colloid) carcinoma

Mucinous cystadenocarcinoma

Signet ring adenocarcinoma

Clear cell adenocarcinoma

Minimally Invasive adenocarcinoma

Nonmucinous

Mucinous

Squamous cell carcinoma

Keratinising Squamous cell carcinoma

Non Keratinising Squamous cell carcinoma

Basaloid

Neuroendocrine tumors

Small cell carcinoma

Combined small cell carcinoma

Large cell carcinoma

Large cell neuroendocrine carcinoma

Combined large cell neuroendocrine carcinoma

Basaloid carcinoma

Lymphoepithelioma-like carcinoma

Clear cell carcinoma

Large cell carcinoma with rhabdoid phenotype

Adenosquamous carcinoma

Sarcomatoid carcinoma

Pleomorphic carcinoma

Spindle cell carcinoma

Giant cell carcinoma

Carcinosarcoma

Pulmonary blastoma

Carcinoid tumour

Typical carcinoid

Atypical carcinoid

Salivary gland tumours

Mucoepidermoid carcinoma

Adenoid cystic carcinoma

Epithelial-myoepithelial carcinoma

Preinvasive lesions

Squamous carcinoma in situ

Atypical adenomatous hyperplasia

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

Mesenchymal tumors

Epithelioidhaemangioendothelioma

Angiosarcoma

Pleuropulmonaryblastoma

Chondroma

Congenial peribronchialmyofibroblastic tumor

Diffuse pulmonary lymphangiomatosis

Inflammatory myofibroblastic tumor

Lymphangioleiomyomatosis

Synovial sarcoma

Monophasic , Biphasic

Pulmonary artery sarcoma

Pulmonary vein sarcoma

Benign epithelial tumors

Papillomas

Squamous cell papilloma

Exophytic

Inverted

Glandular papilloma

Mixed squamous cell and glandular papilloma

Adenomas

Alveolar adenoma

Papillary adenoma

Adenomas of the salivary gland type

Mucous gland adenoma

Pleomorphic adenoma

Others

Mucinous cystadenoma

Lymphohistiocytic tumours

Marginal zone B-cell lymphoma of the MALT type

Diffuse large B-cell lymphoma

Lymphomatoid granulomatosis

Langerhans cell histiocytosis

Miscellaneous tumors

Harmatoma

Sclerosing hemangioma

Clear cell tumors

Germ cell tumors

Teratoma, mature

Immature

Other germ cell tumors

Intrapulmonary thymoma

Melanoma

Metastatic tumors

ANNEXURE IV

KEY TO MASTER CHART

HPE NO – Histopathological Examination Number

SEX:

M - Male

F – Female

SMOKING HISTORY

N- No

Y- Yes

SITE OF LESION:

R – Right lobe, L – Left lobe

RU –Right Upper Lobe

RM- Right Middle Lobe

RL- Right Lower Lobe

LU- Left Upper Lobe

LL- Left Lower Lobe

LH- Left Hilum

CYTOLOGY:

POS – Positive for Malignancy

NEG – Negative for Malignancy

NA- Not Applicable

HPE DIAGNOSIS:

ADC: Adenocarcinoma

SCC: Squamous cell carcinoma

NSCLC-NOS: Non Small Cell Lung Carcinoma-not otherwise Specified

SC- Small Cell Carcinoma

NT- No Tumor

IHC- Immunohistochemistry

EGFR – Epidermal growth factor receptor

ANNEXURE - V

MASTER CHART A

(Patient Details)

S.NO	HPE NO.	AGE	SEX	SMOKING	SITE OF LESION	CYTOLOGY	HPE DIAGNOSIS
1	S1754/15	46	M	N	RM	NA	ADC
2	S1760/15	52	M	Y	LL	NEG	SCC
3	S1981/15	48	M	Y	RU	NA	SCC
4	S2112/15	55	F	N	LL	NEG	NT
5	S2158/15	64	F	N	LU	NEG	SCC
6	S2189/15	60	M	N	RU	NA	ADC
7	S2308/15	55	M	Y	LU	NEG	NT
8	S2371/15	65	M	Y	LL	POS	SCC
9	S2456/15	70	M	N	LU	NA	ADC
10	S20/16	66	M	Y	RU	NA	SCC
11	S76/16	72	M	Y	LU	NEG	SCC
12	S100/16	64	F	N	LU	NEG	NSCLC-NOS
13	S142/16	58	M	Y	RM	POS	SCC
14	S156/16	62	F	N	LU	NEG	SCC
15	S164/16	58	M	Y	LH	POS	SCC
16	171/16	65	M	Y	LL	NA	SCC
17	S188/16	56	F	N	RL	NA	SCC
18	S189/16	61	M	Y	RU	NEG	ADC
19	S248/16	50	M	Y	RM	NA	NSCLC-NOS
20	S278/16	40	M	Y	RL	NA	SCC
21	S308/16	35	F	N	LU	NA	NT
22	S335/16	55	M	Y	RU	NEG	SCC
23	S377/16	55	M	N	LU	NA	ADC
24	S399/16	55	M	Y	RM	POS	SCC

25	S417/16	63	M	Y	LL	NEG	NT
26	S460/16	50	M	Y	LL	NEG	SCC
27	S467/16	63	M	N	LU	NEG	NT
28	S686/16	66	M	N	RU	NA	NSCLC-NOS
29	S734/16	60	M	Y	RU	NA	SCC
30	S764/16	54	M	Y	LL	NA	NT
31	S767/16	58	M	Y	LU	NA	NT
32	S794/16	55	M	Y	RL	NEG	SCC
33	S984/16	45	F	N	RM	NEG	SCC
34	S986/16	67	M	Y	LL	NEG	SCC
35	S987/16	56	M	N	RH	NEG	ADC
36	S995/16	70	M	Y	RU	NA	SCC
37	S1021/16	62	M	Y	LL	NEG	SCC
38	S1029/16	20	M	Y	LU	NA	SC
39	S1030/16	65	M	Y	LU	NEG	SCC
40	S1043/16	50	M	Y	RL	NEG	NSCLC-NOS
41	S1083/16	65	M	Y	LH	NEG	SCC
42	S1104/16	65	F	N	LU	NEG	SCC
43	S1128/16	50	F	N	LU	POS	ADC
44	S1150/16	65	F	N	RU	POS	SCC
45	S1151/16	55	M	Y	LU	POS	SCC
46	S1190/16	42	M	N	RU	NA	ADC
47	S1200/16	45	M	Y	LL	NEG	SCC
48	S1250/16	62	F	N	RL	NEG	ADC
49	S1301/16	50	M	Y	LU	NA	ADC

50	S1304/16	61	M	N	RU	NEG	ADC
51	S1311/16	76	M	Y	LU	NEG	NSCLC-NOS
52	S1327/16	74	M	N	RL	NEG	ADC
53	S1335/16	75	F	N	LL	NEG	SCC
54	S1362/16	48	M	Y	RM	NA	SC
55	S1375/16	65	M	Y	LL	POS	SCC
56	S1376/16	60	M	Y	RH	POS	SCC
57	S1377/16	70	F	N	RL	POS	ADC
58	S1378/16	70	M	N	RL	NA	ADC
59	S1387/16	55	M	Y	RU	NA	ADC
60	S1401/16	62	M	Y	RU	NA	NSCLC-NOS
61	S1442/16	61	M	Y	LL	POS	SCC
62	S1464/16	65	M	Y	RU	NEG	NT
63	S1469/16	40	M	Y	RL	NA	SCC
64	S1470/16	42	M	N	RU	NEG	NT
65	S1505/16	63	F	N	LU	NEG	ADC
66	S1511/16	60	M	Y	LL	NA	SCC
67	S1536/16	45	M	Y	RH	NEG	NT
68	S1537/16	60	M	Y	RU	POS	NSCLC-NOS
69	S1539/16	50	M	Y	RL	NEG	NT
70	S1552/16	59	M	N	RU	NA	ADC
71	S1587/16	60	F	N	LU	POS	SCC
72	S1641/16	42	M	Y	RH	NEG	SC
73	S1642/16	51	M	Y	RL	NEG	ADC
74	S1643/16	48	M	N	LU	NEG	NSCLC- NOS

75	S1658/16	67	M	Y	RU	NA	SCC
76	S1675/16	48	M	Y	LU	NA	SCC
77	S1677/16	50	M	Y	RU	POS	SCC
78	S1699/16	51	M	N	RM	NA	SCC
79	S1725/16	65	F	N	RM	NEG	ADC
80	S1972/16	38	F	N	LU	NEG	ADC
81	S2040/16	56	M	Y	LH	NEG	SCC
82	S2041/16	65	M	Y	RU	NEG	SCC
83	S2060/16	48	M	Y	LU	NEG	NSCLC- NOS
84	S2110/16	65	M	Y	LU	NEG	SCC
85	S2169/16	50	M	N	LU	NA	SCC
86	S2188/16	46	M	Y	LL	POS	SCC
87	S2236/16	55	M	Y	LL	POS	SCC
88	S93/17	50	M	N	RU	NA	ADC
89	S160/17	65	M	Y	LL	NA	SCC
90	S178/17	65	M	N	RL	POS	SCC
91	S181/17	70	M	Y	LU	NEG	SCC
92	S346/17	57	M	Y	RU	NEG	SCC
93	S389/17	60	F	N	LL	NA	NT
94	S390/17	45	M	Y	RL	NEG	SCC

95	S675/17	47	F	N	RM	NA	SCC
96	S870/17	65	M	Y	RU	NEG	SCC
97	S879/17	49	M	N	LU	NEG	SCC
98	S880/17	62	M	Y	RL	NEG	NSCLC- NOS
99	S886/17	70	F	N	RU	POS	SCC
100	S1045/17	53	M	Y	LL	NA	ADC
101	S1089/17	60	M	Y	LU	POS	SCC
102	S1147/17	70	M	Y	RM	NEG	NSCLC-NOS
103	S1305/17	60	M	Y	LU	NA	SCC
104	S1329/17	58	M	N	LU	POS	ADC
105	S1347/17	76	M	Y	RU	NA	SCC
106	S1356/17	55	M	Y	LL	NEG	NSCLC-NOS
107	S 1417/17	62	M	Y	LL	NEG	NT
108	S1419/17	55	F	N	RU	NEG	NT

ANNEXURE - V

MASTER CHART B

(EGFR - IHC)

S.NO	BIOPSY NO	AGE	SEX	HPE DIAGNOSIS	EGFR POSITIVITY	GRADING OF EGFR
1	S1754/15	46	M	ADC	POS	3+
2	S100/16	64	F	NSCLC-NOS	NEG	NEG
3	S189/16	61	M	ADC	NEG	NEG
4	S686/16	66	F	NSCLC-NOS	POS	2+
5	S984/16	45	F	SCC	POS	1+
6	S1043/16	50	M	NSCLC-NOS	POS	3+
7	S1150/16	65	F	SCC	NEG	NEG
8	S1151/16	55	M	SCC	POS	2+
9	S1250/16	62	F	ADC	POS	3+
10	S1304/16	70	F	ADC	POS	3+
11	S1335/16	75	F	SCC	NEG	NEG
12	S1401/16	62	F	NSCLC-NOS	NEG	NEG
13	S1505/16	63	F	ADC	POS	2+
14	S1537/16	60	M	NSCLC-NOS	POS	1+
15	S1552/16	59	M	ADC	POS	3+
16	S1587/16	60	F	SCC	NEG	NEG
17	S1643/16	48	M	NSCLC-NOS	NEG	NEG
18	S1658/16	67	M	SCC	POS	2+
19	S1725/16	65	F	ADC	POS	3+
20	S1972/16	38	F	ADC	POS	2+
21	S2060/16	48	M	NSCLC-NOS	NEG	NEG
22	S93/17	50	M	ADC	POS	3+
23	S346/17	57	M	SCC	POS	3+
24	S675/17	47	F	SCC	POS	2+
25	S880/17	62	M	NSCLC-NOS	NEG	NEG
26	S886/17	70	F	SCC	NEG	NEG
27	S1045/17	53	M	ADC	POS	3+
28	S1147/17	70	M	NSCLC-NOS	POS	3+
29	S1347/17	76	M	SCC	POS	2+
30	S1356/17	55	M	NSCLC-NOS	NEG	NEG

ANNEXURE - VI

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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.C.Sofia Tamilarasi
Course	:	PG in MD., Pathology
Period of Study	:	2015-2018
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Histopathological analysis of lung tumors and study of expression EGFR with immunehistochemical markers
Ethical Committee as on	:	17.03.2017

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

M.Shan
Member Secretary

Prof.Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc (Hon)
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This is to certify that this dissertation work titled "**HISTOPATHOLOGICAL ANALYSIS OF LUNG TUMORS AND STUDY OF EXPRESSION OF EGFR WITH IMMUNOHISTOCHEMICAL MARKERS"** of the candidate **DR.C.SOFIA TAMILARASI** with registration Number **201513102** for the award of **M.D.,** in the branch of **PATHOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **3** percentage of plagiarism in the dissertation.

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